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Protocol for the Analysis of 2,3,7,8-Tetrachlorodibenzo-p-Dioxin by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry

Midwest Research Inst., Kansas City, MO

Prepared for

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16. ABSTRACT

An analytical protocol for the determination of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and total TCDDs in soil, sediment and aqueous samples using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) was developed using the best features of several candidate methods and input from experts in the field. Preliminary tests led to refinements of the chromatographic cleanup procedures and corresponding changes in the protocol. A final single-laboratory evaluation of the refined protocol, consisting of triplicate analyses of five solid and five aqueous samples showed that the method is useful for the determination of 2,3,7,8-TCDD and total TCDDs at concentrations from 10 to 200 pg/g (ppt) in soils and 100 to 2,000 pg/L (ppq) in aqueous samples. Based on the data generated and on the evaluation of several options, parts of the protocol were modified at the EMSL-LV to lower the quantitation limit for TCDD to 2 ppt in soil/sediments and to 20 ppq in aqueous samples.

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PREFACE

This report describes the activities completed as part of a single-laboratory evaluation of a high-resolution gas chromatography/high-resolution mass spectrometry method for the determination of tetrachlorodibenzo-p-dioxins in water, soil, and sediment samples. The work described in this report was completed at the Midwest Research Institute under contract to Viar and Company (Special Analytical Services SAS 1576X) for the U.S. Environmental Protection Agency, Environmental Monitoring Systems Laboratory, Quality Assurance Division, Las Vegas, Nevada. The revision of the protocol to allow for lower quantitation limits for tetrachlorodibenzo-p-dioxins was carried out at the Environmental Monitoring Systems Laboratory-Las Vegas.

This report was prepared with assistance from M. McGrath. The authors acknowledge the technical project monitor, W. F. Beckert, as well as R. K. Mitchum and S. Billets of the Environmental Monitoring Systems Laboratory-Las Vegas and, especially, Y. Tondeur of the Environmental Research Center, University of Nevada, Las Vegas for guidance provided during this study.

ABSTRACT

This report provides the results of the single-laboratory evaluation of a high-resolution gas chromatography/high-resolution mass spectrometry method for the determination of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and total tetrachlorodibenzo-*p*-dioxins at concentrations ranging from 10 to 200 pg/g (ppt) in soils and 100 to 2,000 pg/L (ppq) in water. The report summarizes the data for the precision and accuracy of triplicate measurements of five solid and five aqueous samples. The results indicate that the method is capable of generating accurate and precise data within the concentration limits specified above and within absolute recoveries of 40 to 120 percent with 50 percent precision. An attempt to reach a quantitation limit for TCDD of 2 ppt (or less) for soil and 20 ppq (or less) for aqueous samples was not successful. Based on the data generated during this study and based on discussions at the Environmental Monitoring Systems Laboratory-Las Vegas, the Environmental Monitoring Systems Laboratory-Las Vegas revised certain parts of the protocol to lower the quantitation limit for tetrachlorodibenzo-*p*-dioxins to 2 ppt in soil and 20 ppq in water samples.

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SECTION 1

INTRODUCTION

The U.S. Environmental Protection Agency's (EPA) strategy for dealing with dioxin requires the development and validation of an analytical method capable of achieving detection of the tetrachlorodibenzo-p-dioxins (TCDD), specifically 2,3,7,8-TCDD, at the parts-per-trillion (ppt) level in soil and sediment and parts-per-quadrillion (ppq) level in water.[1] This validated method will be used by qualified contract laboratories to extend the analytical capabilities for such analyses to all EPA regional and program offices.

This report deals specifically with the single-laboratory evaluation of a high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) analysis method for TCDDs in soil, sediment, and water. The method (Appendix A) is intended to provide quantitative determination of TCDD at levels of 10 to 200 pg/g (soil and sediment) and 100 to 2,000 pg/L (water) at a mass resolution of 10,000. This single-laboratory evaluation has been completed as part of the validation process recommended by EPA.[2]

The proposed method was prepared after several candidate methods were reviewed and their best features were selected. After peer review, the proposed method was refined for completeness, technical accuracy, clarity, and regulatory applicability. The single-laboratory evaluation of the proposed analytical method has been accomplished through three tasks. The first task involved preliminary performance testing of the method using TCDD-contaminated soils and TCDD-spiked aqueous samples. The results of this study indicated that the proposed method required modification to achieve the target method detection limits and the accuracy and precision criteria. The second task focused on ruggedness testing of the chromatographic cleanup procedures. The results of this study were used to modify the proposed method. This report is focused on the results of the triplicate analysis of five solid and five aqueous samples completed under the third task of the evaluation, using the modified method.

Section 2 of this report summarizes the conclusions based on the single-laboratory evaluation of this method using TCDD-contaminated soils and TCDD-spiked aqueous samples. Section 3 presents recommendations that should be considered for inclusion in the method before proceeding with collaborative testing. Section 4 presents some specific experimental conditions, and Section 5 summarizes the analytical data for the triplicate analysis of four soil, one fly ash, and five aqueous samples completed in the third task of the single-laboratory evaluation. Triplicate analyses of a 1-pg/µL calibration solution did not give satisfactory results. In order to achieve a quantitation limit of 2 ppt for soil (using a 10-g sample) and

20 ppq for water (using a 2.0-L sample), the protocol evaluated in this study was modified. The rationale for the modifications and the revised protocol are included as Appendix B.

SECTION 2

CONCLUSIONS

The single-laboratory evaluation of the analytical method for the determination of 2,3,7,8-TCDD in soil and aqueous samples demonstrates that the method as described is capable of achieving the target detection limits of 10 pg/g (ppt) for soils and 100 pg/L (ppq) for water.

The relative response factors (RRF) determined for native 2,3,7,8-TCDD versus the internal standard $^{13}C_{12}$-2,3,7,8-TCDD, and the RRF of the internal standard versus the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD over the five-point concentration calibration curve demonstrate that the HRGC/HRMS method maintains a linear response for 2,3,7,8-TCDD from 10 to 200 ppt for soils and 100 to 2,000 ppq for water.

The results of the analysis of spiked aqueous samples demonstrate that internal standard (isotope dilution) quantitation provides an accurate measurement of 2,3,7,8-TCDD. The accuracy of the 2,3,7,8-TCDD measurement for triplicate analysis of four water samples spiked at various concentrations was quite good. The accuracy of measurement for 2,3,7,8-TCDD averaged 104 percent for three aqueous matrices prepared as laboratory matrix spikes. The absolute recovery of the internal standard $^{13}C_{12}$-2,3,7,8-TCDD did not significantly affect the accuracy of the 2,3,7,8-TCDD determination. The precision of the analyses for 2,3,7,8-TCDD ranged from 3.6 to 16 percent for replicate analyses of the five aqueous samples. The precision of the triplicate analyses of the soil samples was somewhat higher than determined for aqueous samples. The precision of triplicate analyses of the four soil samples ranged from 19 to 50 percent. The difference in precision from that of the aqueous samples may be attributable to the potential for TCDD adsorption on the soil samples.

The results from the analyses of soil and aqueous samples spiked with additional TCDD isomers demonstrate that the internal standard quantitation gives good estimates of total TCDD values. The accuracy of the analyses of fortified distilled water and influent and effluent wastewaters averaged 101 ± 14 percent for five TCDD isomers. The accuracy of the measurements of these isomers for the four fortified soil samples averaged 87 ± 24 percent.

The results of the analyses demonstrate that the requirements for absolute recovery of the internal standard (40 to 120 percent) and precision of replicate analyses (RPD < 50 percent) can be achieved for relatively clean samples.

The sample matrix can severely impact the performance of the analytical method. This is evidenced by the consistent low recovery of the internal standard from the fifth aqueous sample, an industrial wastewater, and from a fly ash sample. The low recovery from the industrial wastewater is possibly due to the effect of coextractants on the elution sequence from alumina. The low recoveries observed for the fly ash sample, on the other hand, may be attributed to adsorption by the sample matrix.

One of the most critical variables in the analytical method is the completeness of removal of the benzene from the extract before proceeding with the acidic alumina column fractionation. The cleanup column ruggedness testing experiments demonstrated that the recoveries of 2,3,7,8-TCDD and the other TCDDs are affected by the presence of benzene in the alumina column fractionation step.

The analyst must be aware of the potential problem of interferences arising from background contamination. For example, the 1,3,6,8- and 1,3,7,9-TCDD isomers were present in the fortified field blanks in this work. From other referenced activities it becomes clear that these isomers may present problems in other laboratories as well. The fortified field blanks are important tools in assessing the background contamination problems over time.

Although the 1.0-pg/μL standard did not yield satisfactory results in this study, due to unacceptable ion ratios, the response factors are within the established curve. The data for the triplicate analyses of the 1.0-μg/μL standard demonstrate that the characteristic ions for TCDD were greater than 20:1 for the m/z 322 S/N and approximately 10:1 for m/z 259 S/N. Thus, it should be possible to extend the detection limit to 1 pg/μL if an allowance for abundance ratios based on ion statistical errors is incorporated.

Based on the column performance and bleed characteristics, the column of choice for the analysis for TCDD at ppt (for soils and sediments) and ppq (for water) levels appears to be the 50-m CP-Sil 88 with a 0.2-μm film thickness. To preserve the performance characteristics of the HRGC columns, an injection technique that excludes any air is highly recommended.

SECTION 3

RECOMMENDATIONS

1. Mass measurement accuracy should properly be determined relative to the lock mass (if any), rather than m/z 254.9856, because it is that relationship which will determine how accurately the masses of the TCDD ions will be measured.

2. It is recommended that the chromatographic resolution check be performed on the summed ion chromatograms of m/z 259 + m/z 320 + m/z 322. This yields a chromatogram which is less noisy and more representative of the true column performance.

3. The 5 percent peak width criterion for mass resolution should be the selected mass/1,000 mmu rather than 31.9 mmu because the protocol allows peaks other than m/z 319 to be used for resolution measurement (e.g., 31.7 mmu if m/z 317 is used).

4. It is recommended that the mass measurement accuracy be recorded and reported along with the resolution check summary table.

5. The addition of the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD should be achieved by using a spike volume of 25 to 50 µL rather than 5 µL to minimize errors resulting from volume measurement.

6. The recommended temperature program settings in the method should be converted to those presented in the experimental section of this report. These conditions were established for analysis with tridecane as the solvent.

7. Lower limits of detection can be achieved by allowing the analyst to concentrate the final extract to as low as 10 µL. It may be necessary to use the smaller final volume with other HRMS instruments to achieve the same levels of detection.

8. The method should recommend several techniques to break up emulsions resulting from extraction of aqueous samples. In this evaluation the emulsion phase was put through a column packed with glass wool, which was then rinsed with additional methylene chloride. Other options might include stirring or centrifugation of the emulsion phase.

9. The method should specify the procedure to deal with aqueous samples containing high levels of suspended solids. In this study it was

necessary to centrifuge the soil extract sample before proceeding with the extraction.

10. It is highly recommended that the method be modified such that the benzene extract is completely exchanged to hexane prior to cleanup on the silica column since this is apparently one of the most critical factors leading to successful sample analysis.

11. It may be worthwhile to evaluate a cleanup procedure in which the charcoal column precedes the alumina column as a means to improve method recovery.

SECTION 4

EXPERIMENTAL PROCEDURES

SAMPLE DESCRIPTION

Five solid samples were provided by the Environmental Monitoring Systems Laboratory-Las Vegas (EMSL-LV) to the Midwest Research Institute (MRI) for analysis for 2,3,7,8-TCDD and total TCDD using the analytical method in Appendix A. A description of the five solid samples and the estimated 2,3,7,8-TCDD concentrations from previous analyses by an independent laboratory are provided in Table 1. Each sample was analyzed in triplicate as specified in the protocol. One of the triplicate samples for each soil sample was spiked with the seven TCDD isomers (1,3,6,8-; 1,3,7,9-; 1,2,3,7-; 1,2,3,8-; 1,2,3,4-; 1,2,7,8-; and 1,2,8,9-TCDD) at approximately 10 times the estimated level of 2,3,7,8-TCDD specified in Table 1.

Five aqueous samples were generated for the evaluation of the analytical method at the ppq detection level. Table 2 presents a description of each water type and lists the fortification levels of 2,3,7,8-TCDD and seven additional TCDD isomers (1,3,6,8-; 1,3,7,9; 1,2,3,7-; 1,2,3,8-; 1,2,3,4-; 1,2,7,8-; and 1,2,8,9-TCDD) in each sample.

The influent and effluent wastewater samples were collected from a sewage treatment facility in metropolitan Kansas City, Missouri. The industrial wastewater was obtained from a holding pond within a hazardous waste area that was known to be highly contaminated with PCBs and possibly other chlorinated aromatic compounds (chlorobenzenes). This aqueous sample was very acidic (pH < 1) and was dark in color.

The soil extract was prepared from 30 g of a soil sample, Hyde Park 002 (H2), and 1 gallon of distilled water. The mixture was stirred constantly (at least 24 hrs) until just prior to subsampling of 1.0-L aliquots.

SAMPLE PREPARATION

All samples listed in Tables 1 and 2 were extracted and analyzed in triplicate according to the protocol provided in Appendix A. As indicated in Tables 1 and 2, one aliquot of each sample matrix was fortified with additional TCDD isomers, which represent the compounds that elute first (1,3,6,8-TCDD), last (1,2,8,9-TCDD), and within the approximate retention window of 2,3,7,8-TCDD (1,2,3,7-; 1,2,3,8-; and 1,2,3,4-TCDD) from the HRGC columns used for sample analysis.

TABLE 1. SOLID SAMPLES USED FOR HRGC/HRMS METHOD EVALUATION

EPA sample no.	Matrix	Approximate sample size[a]	Estimated 2,3,7,8-TCDD concentration (ppt)[b]	Spike level (ppt) of TCDD isomers[c]
B25-Piazza Road (B5)	Soil	10 g	50	100
Hyde Park 001 (H1)	Soil	10 g	70	140
B52-Shenandoah (B1)	Soil	1 g	360	720
Hyde Park 003 (H3)	Soil	1 g	1,700	1,700
RRAI-5,7,8 (FA)	Fly ash	10 g	NR[d]	e

---

[a] Approximate sample size of each replicate sample.

[b] Estimated level of endogenous 2,3,7,8-TCDD reported to MRI by W. Beckert in letters dated April 19, 1985 and August 30, 1985.

[c] Approximate fortification level of each of seven additional TCDD isomers.

[d] No estimate of 2,3,7,8-TCDD concentration was reported.

[e] Additional TCDD isomers were not spiked into this matrix.

TABLE 2. AQUEOUS SAMPLES USED FOR HRGC/HRMS METHOD EVALUATION

Sample type	Approximate sample size[a]	Fortification level of 2,3,7,8-TCDD (ppq)	Fortification level of TCDD isomers (ppq)[b]
Distilled water (DW)	1.0 L	250	500
POTW influent (IWW)	1.0 L	500	1,000
POTW effluent (EWW)	1.0 L	1,000	2,000
Industrial wastewater (IND)	1.0 L	500	1,000
Hyde Park 002; soil extract (H2W)	1.0 L	c	c

---

[a] Approximate sample size of each replicate sample.

[b] Approximate fortification level of each of seven additional TCDD isomers.

[c] This aqueous sample was not fortified with TCDD isomers.

All samples were fortified with 500 pg $^{13}C_{12}$-2,3,7,8-TCDD in 1.5 mL acetone. The solid samples were extracted continuously for 24 hr in a Soxhlet apparatus with benzene and the 1.0-L aqueous samples were batch-extracted using 2.0-L separatory funnels and three 60-mL portions of methylene chloride. The extractions of the influent wastewater (IWW) and effluent wastewater (EWW) and the soil extract (H2W) were complicated by the formation of emulsions. In each case, the emulsion was removed by passing the methylene chloride and emulsion layer through a column of glass wool prerinsed with methylene chloride. The extract and resulting aqueous layers were collected in a sample bottle and the glass wool plug was rinsed with an additional 10 mL methylene chloride. Following the complete extraction of the aqueous sample, the contents of the bottle were transferred to a clean 250-mL separatory funnel and the methylene chloride was removed from the aqueous phase that was transferred with the emulsion. All extracts were concentrated with Kuderna-Danish evaporators and nitrogen evaporation to approximately 1.0 mL. Each extract was taken through the entire cleanup procedure including the acidic silica, acidic alumina, and Carbopak C as specified in the protocol (Appendix A). The HRGC/HRMS analysis of each extract was completed as specified below.

REAGENTS

All solvents for extraction and cleanup were obtained as "Burdick and Jackson distilled-in-glass" quality. The tridecane (99 percent purity) was obtained from Aldrich (TS, 740-1). The chromatographic materials, acidic alumina (100-200 mesh AG-4, Biorad Laboratories 132-1340), silica (70-230 mesh Kieselgel 60, EM Reagent, American Scientific Products C5475-2), sodium sulfate, potassium carbonate, Celite 545® (Fisher Scientific Company), and the silanized glass wool and Carbopak C (80-100 mesh Supelco 1-1025) were prepared for use as specified in Section 7 of the protocol (Appendix A).

Table 3 provides the sources of standards used to prepare the calibration solutions, sample fortification solutions, recovery standard spiking solution, internal standard spiking solutions, field fortification solutions, and TCDD isomer fortification solutions.

Table 4 is a summary of the concentration calibration standards prepared for the HRGC/HRMS method evaluation. These standards were prepared as specified in the protocol (Appendix A). The standard HRCC6 was included in the final evaluation of the HRGC/HRMS method as a means to demonstrate the lower limit of detection under optimum instrumental conditions.

HRGC/HRMS INSTRUMENTATION

Sample extracts and calibration standards were analyzed using a Carlo Erba Mega Series gas chromatograph (GC) which was coupled to a Kratos MS50 TC double-focusing mass spectrometer (MS). The GC/MS interface was simply a direct connection of the GC column to the ion source via a heated interface oven. A Finnigan 2300 Incos data system was used for data acquisition and processing.

TABLE 3. TCDD ISOMERS USED FOR HRGC/HRMS METHOD EVALUATION

Isomer	Stock concentration	Source	Standard code
2,3,7,8-TCDD	7.87 ± 0.79 µg/mL	EPA QA Reference Materials	20603
$^{13}C_{12}$-2,3,7,8-TCDD	50 ± 5 µg/mL	Cambridge Isotope Laboratories	R00201 (Lot AWN-1203-65)
1,2,3,4-TCDD	2.7 mg/mL	Cambridge Isotope Laboratories	ED-915C (Lot 6578)
$^{13}C_{12}$-1,2,3,4-TCDD	50 ± 5 µg/mL	Cambridge Isotope Laboratories	R00212 (Lot AWN-1203-93)
1,3,6,8-/1,3,7,9-TCDD	0.82 mg/mL	Cambridge Isotope Laboratories	ED-913C (Lot F2086)
1,2,3,7-/1,2,3,8-TCDD	0.5 mg/mL	Cambridge Isotope Laboratories	ED-905C (Lot 7371)
1,2,7,8-TCDD	0.39 mg/mL	Cambridge Isotope Laboratories	ED-915C (Lot 7184)
1,2,8,9-TCDD	1.46 mg/mL	Cambridge Isotope Laboratories	ED-916C (Lot MLB-682-26)
Column performance standard[a]	10 µg/mL	Cambridge Isotope Laboratories	ED-908 (Lot No. R00215)

[a] Mixture of TCDD isomers including 2,3,7,8-; 1,2,3,4-; 1,2,3,7-/1,2,3,8-; 1,2,7,8-; and 1,4,7,8-TCDD.

TABLE 4. COMPOSITION OF CONCENTRATION CALIBRATION SOLUTIONS (pg/µL)

	Recovery standard $^{13}C_{12}$-1,2,3,4-TCDD	Analyte 2,3,7,8-TCDD	Internal standard $^{13}C_{12}$-2,3,7,8-TCDD
HRCC1	2.5	2.5	10.0
HRCC2	5.0	5.0	10.0
HRCC3	10.0	10.0	10.0
HRCC4	20.0	20.0	10.0
HRCC5	40.0	40.0	10.0
HRCC6[a]	1.0	1.0	10.0

[a] This solution is not specified in the analytical method in Appendix A.

The HRGC/HRMS operating conditions used in the final phase of this work are summarized in Table 5. The GC operating conditions recommended in the protocol were not used for these analyses for three reasons. First, the TCDDs have rather long retention times, and the solvent (tridecane) boils at 235°C. Thus no benefit could be realized with a low initial temperature. Second, past experience at MRI has indicated that 200°C is an acceptable starting temperature for these types of analyses when tridecane is used as a solvent. Finally, since the CP-Sil 88 and SP-2330 phases are both very polar and thinly coated, it has been recommended that they not be subjected to rapid heating or cryogenic cooling to prevent thermal shock to the column.[3]

The MS was tuned daily to yield a resolution of at least 10,000 (10 percent valley) and optimal response at m/z 254.986. This step was followed by calibration of an accelerating voltage scan beginning at m/z 254 (typical calibration range was 255 to 605 amu). Other voltage scans from the same data file were then used to establish and document both the resolution at m/z 316.983 and the mass measurement accuracy at m/z 330.979.

MASS MEASUREMENT ACCURACY

For this work, mass measurement accuracy was measured relative to PFK m/z 254.986, as required by the protocol, by applying the mass correction, Δm, to the entire spectrum, which yields an error of 0 ppm at m/z 254.986. In this way, it was possible to meet routinely the 5 ppm accuracy criterion at m/z 330.979. However, if a lock mass other than 254.986 is used, the mass measurement accuracy should be measured relative to that lock mass, since it is that peak which is used to maintain magnet alignment and will ultimately control the mass measurements during the selected ion monitoring (SIM) experiments.

Mass Resolution

Mass resolution at m/z 316.983 was documented by an output of the Incos PROF program. However, the computer-generated value for resolution was found to be significantly higher than the value measured manually. Thus, the manually determined resolution, which was nearly identical to the value measured by using the peak matching unit, is reported. Closer inspection of the PROF source code revealed that resolution is computed via a statistical method, not as $m/\Delta m$ at 5 percent height. Incos users should therefore be aware of this discrepancy, because the computer-generated value can be as much as 20 percent over the proper value.

Following calibration, the SIM experiment descriptor was updated to reflect the new calibration. Six masses (see Table 5) were monitored by scanning ~ m/10,000 amu over each mass. The total cycle time was kept to 1 sec. The m/z 280.983 ion from PFK was used as a lock mass because it is the most abundant PFK ion within the range of m/z 255 to 334 and therefore permits the use of low partial pressures of PFK, which minimizes PFK interferences at the analytical masses.

TABLE 5. HRGC/HRMS OPERATING CONDITIONS

Mass spectrometer	
Accelerating voltage:	8,000 V
Trap current:	500 μA
Electron energy:	70 eV
Electron multiplier voltage:	2,000 V
Source temperature:	280°C
Resolution:	10,000 (10% valley definition)
Ions monitored	**Nominal dwell times (sec)**
258.930	0.15
319.897	0.15
321.894	0.15
331.937	0.15
333.934	0.15
280.9825 (lock mass)	0.10
Overall SIM cycle time = 1 sec	
Gas chromatograph	
Column coating:	CP-Sil 88
Film thickness:	0.2 μm
Column dimensions:	50 m x 0.22 mm ID
Helium linear velocity:	∼ 25 cm/sec
Helium head pressure:	1.75 kg/cm^2 (25 psi)
Injection type:	Splitless, 45 sec
Split flow:	30 mL/min
Purge flow:	6 mL/min
Injector temperature:	270°C
Interface temperature:	240°C
Injection size:	2 μL
Initial temperature:	200°C
Initial time:	1 min
Temperature program:	200°C to 240°C at 4°C/min

CHROMATOGRAPHIC RESOLUTION

Chromatographic resolution values were measured for the SIM plot of m/z 320. However, it may be advantageous to measure chromatographic resolution from a plot of the sum of m/z 259, 320, and 322. The sum trace has better signal-to-noise ratio (S/N) and peak definition than the SIM plots, which permits a more accurate measurement of resolution.

Selection of the HRGC Column

Three different HRGC columns were evaluated in the course of this project: SP-2330 (60 m x 0.24 mm); DB5 (60 m x 0.22 mm); and CP-Sil 88 (50 m x 0.22 mm). By evaluating the mass spectra of the bleed from each column at 240 to 250°C, it became apparent that the column background may be the limiting factor in achieving the desired detection limit for this method. The DB5 column provided the least amount of background at 250°C, and the SP-2330 had the worst. This coincides with the fact that quantitation at the detection limit (i.e., 2.5 pg/µL) with the SP-2330 column was difficult at best. The CP-Sil 88 column appeared to offer less bleed than the SP-2330 column and indeed does permit more accurate quantitation due to reduced background contribution.

The chromatographic performance afforded by these columns is a further issue, since the column best suited for low detection limits, DB-5, cannot meet the 25 percent valley chromatographic resolution criteria in all cases. Both the SP-2330 and CP-Sil 88 columns can easily resolve the 2,3,7,8-TCDD. However, based on the bleed considerations discussed above, the 50-m CP-Sil 88 column is recommended for the best combination of low bleed and good isomer separation.

It may also be advisable that other HRGC columns (including SP-2340, Silar 10C, and SP-2331) that have been used for 2,3,7,8-TCDD analysis at the 1-ppb soil level be evaluated for background contribution and their application for HRMS analysis at ppt and ppq concentrations.

INJECTION TECHNIQUE

The HRGC column performance can degrade very quickly if proper injection techniques are not used. Specifically, the SP-2330 and CP-Sil 88 phases are very sensitive to O_2 and will decompose rapidly at 200°C if any trace of O_2 is present. Therefore, the common practice of using 1 µL of air to flush the syringe and effect reproducible injections is to be avoided, since even that small amount of air per injection can cause column performance to degrade in less than one week of continued use.

The following injection technique is recommended. First rinse the syringe copiously with isooctane (or other volatile solvent, such as toluene). Dry the syringe by drawing air through it. Pull up and expel several volumes of tridecane until all bubbles are gone, and leave 1 µL of tridecane in the barrel. Finally, pull up 2 µL of the sample solution and inject. This technique has worked very well and yields injection reproducibility comparable to that of the air purge method, without introducing air onto the analytical GC column.

SECTION 5

RESULTS AND DISCUSSION

The primary purpose of any method validation process is to assure that the method under consideration is adequate to meet testing and monitoring requirements.[1] The single-laboratory evaluation of the analytical protocol presented in this report has been preceded by several evaluation and improvement steps. These have included the preparation of a written protocol, technical review of the protocol for completeness, technical accuracy, and clarity; preliminary testing to evaluate performance of the analytical method; and revision and refinement of the written protocol based on the results of the preliminary testing.

Prior to the assessment of the refined protocol presented in Appendix A, the proposed analytical method had been evaluated for performance through the analysis of several duplicate samples. The results of the preliminary evaluation indicated that problems existed in the design and approach to the extract cleanup steps, which greatly affected the method detection limit, accuracy, and precision.

This section presents a summary of the studies that have led to the refinement of the analytical protocol as provided in Appendix A and also summarizes the single-laboratory evaluation of this protocol.

APPROACH TO CLEANUP COLUMN EVALUATION

The initial method evaluation completed under the first task resulted in very low recoveries of the internal standard, $^{13}C_{12}$-2,3,7,8-TCDD, and the accuracy and precision of duplicate sample analyses were poor. After reviewing the data, it was apparent that the problems were the result of poor chromatographic separation in the cleanup columns. The initial protocol involved reducing sample extract volumes to 1.0 mL in benzene, elution through the acidic silica column with hexane, and collection of the total eluent which was then added to the acidic alumina column. The alumina column was further eluted with hexane/20-percent methylene chloride. The eluate was concentrated and cleaned further using a Carbopak C/Celite column, and the TCDDs were eluted with 2 mL toluene.

Column cleanup techniques were revised and further evaluated following the procedures depicted in Figures 1 and 2. The column evaluations were completed with triplicate measurements at three spike levels (0.10, 1.0, and 10 ng) equivalent to 10, 100, and 1,000 ppt of TCDD in solids with several TCDD isomers (2,3,7,8-; 1,3,6,8-; 1,3,7,9-; 1,2,3,4-; 1,4,7,8-; 1,2,3,7-; 1,2,3,8-; and 1,2,8,9-TCDD).

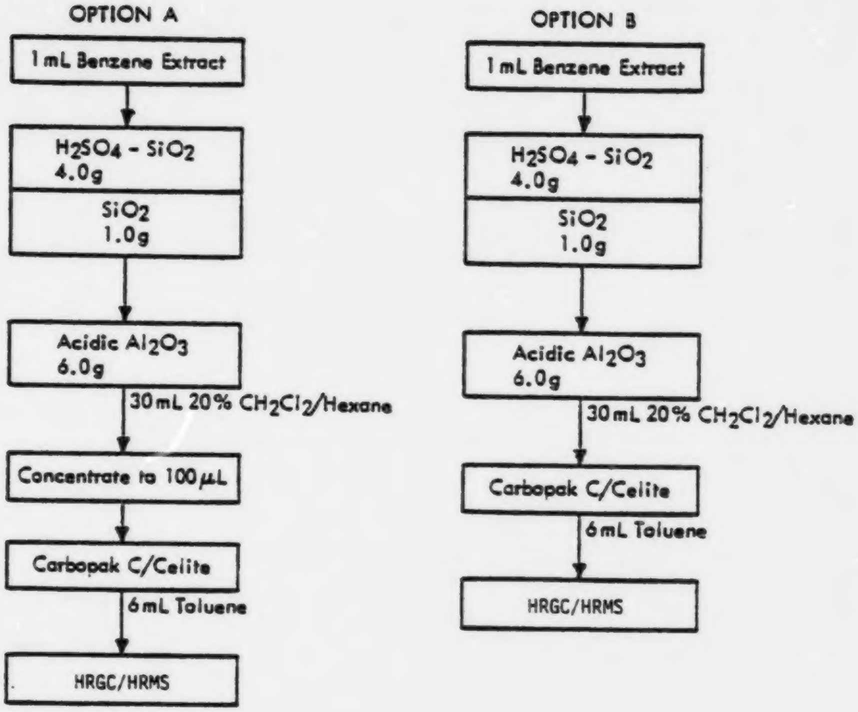

OPTION A
1 mL Benzene Extract
H_2SO_4 - SiO_2 4.0g
SiO_2 1.0g
Acidic Al_2O_3 6.0g
30 mL 20% CH_2Cl_2/Hexane
Concentrate to 100 µL
Carbopak C/Celite
6 mL Toluene
HRGC/HRMS
OPTION B
1 mL Benzene Extract
H_2SO_4 - SiO_2 4.0g
SiO_2 1.0g
Acidic Al_2O_3 6.0g
30 mL 20% CH_2Cl_2/Hexane
Carbopak C/Celite
6 mL Toluene
HRGC/HRMS

Figure 1. Column cleanup procedures specified in the protocol.

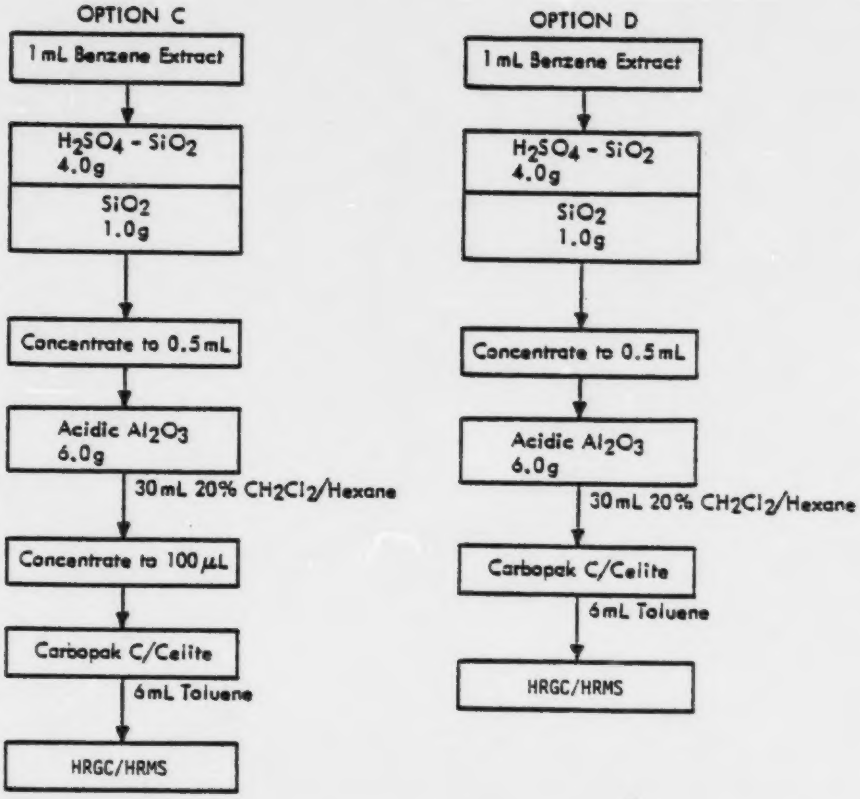

OPTION C
1 mL Benzene Extract
H2SO4 - SiO2 4.0g
SiO2 1.0g
Concentrate to 0.5 mL
Acidic Al2O3 6.0g
30 mL 20% CH2Cl2/Hexane
Concentrate to 100 µL
Carbopak C/Celite
6 mL Toluene
HRGC/HRMS
OPTION D
1 mL Benzene Extract
H2SO4 - SiO2 4.0g
SiO2 1.0g
Concentrate to 0.5 mL
Acidic Al2O3 6.0g
30 mL 20% CH2Cl2/Hexane
Carbopak C/Celite
6 mL Toluene
HRGC/HRMS

Figure 2. Column cleanup procedures proposed by the EMSL-LV.

The TCDD isomers were added to 1-mL portions of benzene and were taken through the four sample cleanup sequences depicted in Figures 1 and 2. One of the replicates for each procedure was also spiked with 100 ng of Aroclor 1260.

The results of the sample analyses are provided in Tables 6 through 9. As noted in Tables 6 and 7, recoveries of the TCDD isomers were low and quite variable for the early eluting isomers 1,3,6,8- and 1,3,7,9-TCDD as compared to 1,2,8,9-TCDD. Recovery of 1,2,8,9-TCDD was still low and variable (approximately 60 percent recovery with an RSD of ~ 20 percent). These results were generated using the procedures specified in the original protocol (see Figure 1). The results of the analyses following the cleanup options A and B demonstrate that accurate quantitation of all TCDD isomers is not possible using only the $^{13}C_{12}$-2,3,7,8-TCDD surrogate standard. The low recoveries measured for options A and B are obviously a result of the presence of benzene in the eluent from the acid-modified silica column that is taken directly through the acidic alumina column.

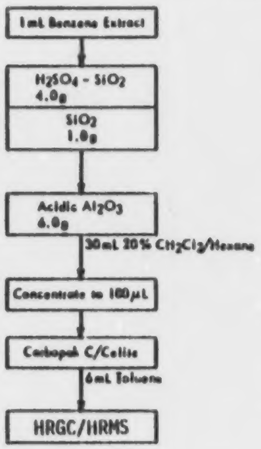
In contrast, options C and D (Tables 8 and 9) demonstrate quantitative recovery of the TCDD isomers. Some background contamination has been noted from the acidic alumina for the 1,3,6,8- and 1,3,7,9-TCDD isomers. This material had previously been prepared by Soxhlet extraction with methylene chloride and activation at 190°C prior to use. As noted in Tables 8 and 9, the average recovery of the other spiked TCDD isomers was greater than 84 percent.

When the recoveries of the different isomers and the $^{13}C_{12}$-2,3,7,8-TCDD are compared, the average relative percent difference ranges from 1 percent for 2,3,7,8-TCDD (Table 3) to 24 percent for 1,2,3,4-TCDD (Table 4). These results demonstrate that either of these cleanup procedures (options C and D) will provide good recovery and reliable quantitation of 2,3,7,8-TCDD and very good estimates of the concentrations of the other TCDD isomers present in the samples. No interferences were observed in the samples spiked with 100 ng Aroclor 1260. The lack of PCB interferences was especially noted in the extracts of samples spiked at 0.10 ng/TCDD isomer.

In addition to the evaluations of the cleanup procedures presented above, the acid-modified silica gel/acidic alumina columns and the Carbopak C/Celite column were evaluated separately. Evaluation of the silica/alumina at the 0.10-ng spike level as shown in Figure 2 resulted in an average recovery of 120 percent for 1,2,3,4-, 1,2,3,7-, 1,2,3,8-, and 1,4,7,8-TCDD; 114 percent for 2,3,7,8-TCDD; 118 percent for $^{13}C_{12}$-2,3,7,8-TCDD; and 118 percent for 1,2,8,9-TCDD. The results for the recovery of 1,3,6,8- and 1,3,7,9-TCDD indicated that some contamination originated from the acidic alumina.

Replicate analyses of the Carbopak C/Celite column at the 0.10-ng spike level resulted in average recoveries of 97 percent for 1,3,6,8-TCDD; 88 percent for 1,3,7,9-TCDD; 81 percent for 1,2,3,4-, 1,2,3,7-, 1,2,3,8-, and 1,4,7,8-TCDD; 75 percent for 2,3,7,8-TCDD; 96 percent for $^{13}C_{12}$-2,3,7,8-TCDD; and 90 percent for 1,2,8,9-TCDD. Elution of the Carbopak C/Celite column with additional toluene beyond 6 mL did not improve recoveries even for the samples spiked at 10 ng/TCDD isomer.

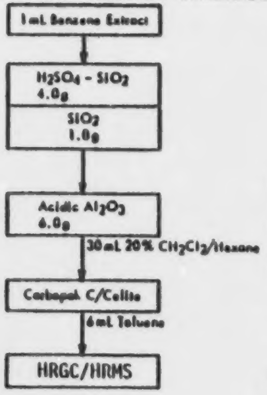
TABLE 6. RECOVERY (%) OF SEVERAL TCDD ISOMERS FROM CLEANUP OPTION A


1mL Benzene Extract
H_2SO_4 - SiO_2 4.0g
SiO_2 1.0g
Acidic Al_2O_3 6.0g
30mL 20% CH_2Cl_2/Hexane
Concentrate to 100µL
Carbopak C/Celite
6mL Toluene
HRGC/HRMS

Spike level	Recovery (%) of TCDD isomer						
	1,3,6,8	1,3,7,9	1,2,3,4/ 1,4,7,8	1,2,3,7/ 1,2,3,8	2,3,7,8	$^{13}C_{12}$-2,3,7,8	1,2,8,9
1 ng	4.6	9.6	21	14	19	27	38
1 ng	12	21	40	33	39	48	64
1 ng[a]	3.1	9.3	28	17	23	31	40
10 ng	12	19	41	31	44	38	76
10 ng	6.2	12	29	20	31	36	61
10 ng[a]	6.3	12	31	20	29	34	59
Mean	7.4	14	32	23	31	36	56
% RSD	51	36	24	34	31	20	26

[a] Sample was also spiked with 100 ng of Aroclor 1260.

TABLE 7. RECOVERY (%) OF SEVERAL TCDD ISOMERS FROM CLEANUP OPTION B


1 mL Benzene Extract
H_2SO_4 - SiO_2 4.0g
SiO_2 1.0g
Acidic Al_2O_3 6.0g
30 mL 20% CH_2Cl_2/Hexane
Carbopak C/Celite
6 mL Toluene
HRGC/HRMS

Spike level	Recovery (%) of TCDD isomer						
	1,3,6,8	1,3,7,9	1,2,3,4/ 1,4,7,8	1,2,3,7/ 1,2,3,8	2,3,7,8	$^{13}C_{12}$-2,3,7,8	1,2,8,9
1 ng	7.6	15	35	14	37	38	48
1 ng	2.4	21	31	17	34	40	52
1 ng[a]	6.4	25	34	22	40	41	57
10 ng	0.9	7.6	14	21	50	44	70
10 ng	1.8	11	36	25	47	48	59
10 ng[a]	11	17	44	31	59	52	78
Mean	5.0	16	32	22	45	44	61
% RSD	79	40	31	28	21	12	19

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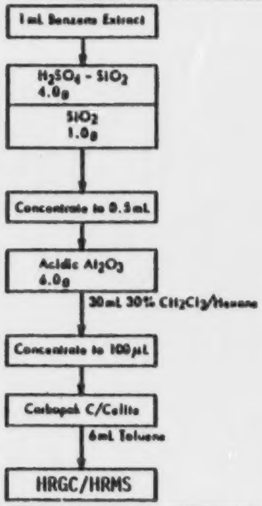
[a] Sample was also spiked with 100 ng of Aroclor 1260.

TABLE 8. RECOVERY (%) OF SEVERAL TCDD ISOMERS FROM CLEANUP OPTION C


1 mL Benzene Extract
$H_2SO_4 - SiO_2$ 4.0g
SiO_2 1.0g
Concentrate to 0.5mL
Acidic Al_2O_3 6.0g
30mL 30% CH_2Cl_2/Hexane
Concentrate to 100µL
Carbopak C/Celite
6mL Toluene
HRGC/HRMS

	Recovery (%) of TCDD isomer						
Spike level	1,3,6,8[a]	1,3,7,9[a]	1,2,3,4/ 1,4,7,8	1,2,3,7/ 1,2,3,8	2,3,7,8	$^{13}C_{12}$-2,3,7,8	1,2,8,9
0.10 ng	300	390	107[b]	c	107	89	81
0.10 ng	310	420	110[b]	c	102	89	84
0.10 ng[d]	340	440	97[b]	c	88	92	91
1.0 ng	158	165	140	116	94	95	89
1.0 ng	155	162	98	96	80	75	80
1.0 ng[d]	168	183	136	120	96	90	94
10.0 ng	171	155	118	116	110	88	107
10.0 ng	157	140	130	130	130	106	122
10.0 ng[d]	130	130	102	99	100	85	110
Mean	210	240	115	112	101	90	95
% RSD	39	54	14	12	14	9.1	15

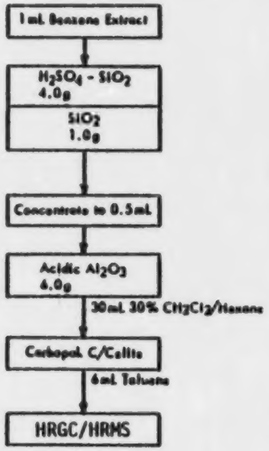
[a] The 1,3,6,8- and 1,3,7,9-TCDD isomers were also noted in reagent blanks from the acidic alumina column. No such interferences were noted from the acidified silica gel or the Carbopak C/Celite column.

[b] Resolution of 1,2,3,4-, 1,2,3,7-/1,2,3,8-, and 1,4,7,8-TCDD was not achieved. This value represents recovery of the four isomers.

[c] Recovery reported with 1,2,3,4-/1,4,7,8-TCDD.

[d] Sample was also spiked with 100 ng of Aroclor 1260.

TABLE 9. RECOVERY (%) OF SEVERAL TCDD ISOMERS FROM CLEANUP OPTION D


1 mL Benzene Extract
H_2SO_4 - SiO_2 4.0g
SiO_2 1.0g
Concentrate to 0.5mL
Acidic Al_2O_3 6.0g
30mL 30% CH_2Cl_2/Hexane
Carbopak C/Celite
6mL Toluene
HRGC/HRMS

Spike level	Recovery (%) of TCDD isomer: 1,3,6,8[a]	1,3,7,9[a]	1,2,3,4/ 1,4,7,8	1,2,3,7/ 1,2,3,8	2,3,7,8	$^{13}C_{12}$-2,3,7,8	1,2,8,9
0.10 ng	147	197	113[b]	c	72	84	70
0.10 ng	290	200	121[b]	c	71	90	71
0.10 ng[d]	260	360	110[b]	c	84	97	e
1.0 ng	90	93	106	103	85	86	53
1.0 ng	180	185	95	121	109	90	108
1.0 ng[d]	135	158	85	79	47	60	63
10.0 ng	81	107	50	104	101	78	84
10.0 ng	114	72	105	108	107	82	118
10.0 ng[d]	126	138	89	95	91	92	112
Mean	158	168	97	101	85	84	85
% RSD	46	51	21	14	23	13	29

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[a] The 1,3,6,8- and 1,3,7,9-TCDD isomers were also noted in reagent blanks from the acidic alumina column. No such interferences were noted from the acidified silica gel or the Carbopak C/Celite column.

[b] Resolution of 1,2,3,4-, 1,2,3,7-/1,2,3,8-, and 1,4,7,8-TCDD was not achieved. This value represents recovery of the four isomers.

[c] Recovery reported with 1,2,3,4-/1,4,7,8-TCDD.

[d] Sample was also spiked with 100 ng of Aroclor 1260.

[e] HRGC/HRMS analysis was interrupted prior to the elution of this isomer.

Three additional experiments were completed to evaluate the efficiency of reverse elution of the carbon column. The Carbopak C/Celite was placed in a 5-mL disposable pipette packed at both ends with glass wool plugs. The column was eluted in one direction for the hexane, cyclohexane/methylene chloride, and the methylene chloride/methanol/benzene mixture. The column was then turned over and eluted with 6 mL toluene. Triplicate analyses at the 0.10 ng/TCDD isomer spike level demonstrated average recoveries of 98 percent for 1,3,6,8-TCDD; 91 percent for 1,3,7,9-TCDD; 104 percent for 1,2,3,4-, 1,2,3,7-, 1,2,3,8-, and 1,4,7,8-TCDD; 116 percent for 2,3,7,8-TCDD; 102 percent for $^{13}C_{12}$-2,3,7,8-TCDD; and 93 percent for 1,2,8,9-TCDD.

FINAL METHOD EVALUATION

Based on the results of the column evaluation study, the analytical method was revised to specify the cleanup procedure presented as Option D in Figure 2. The final protocol, as presented in Appendix A, was then evaluated as described below.

The data presented in Tables 10 through 17 are summaries of the initial column calibration, HRGC and HRMS resolution checks, and the results of the sample analysis.

Calibration

Table 10 summarizes the RRF data for the concentration calibration standards from the initial calibration and the routine monitoring of the RRF values over the time required to complete the sample analyses. The RRF(I) as specified in the protocol is a measure of the response of 2,3,7,8-TCDD versus the internal standard, $^{13}C_{12}$-2,3,7,8-TCDD. The value for RRF(I) varied ± 9.4 percent over the five concentration levels of 2,3,7,8-TCDD ranging from 2.5 pg/μL to 40 pg/μL. The RRF(II) is used to calculate the absolute recovery of the internal standard as compared to the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD. The average RRF(II) was determined to vary by ± 19.3 percent over the calibration curve. The variability of the RRF(I) and RRF(II) were determined to be less than ± 10 percent and ± 18 percent, respectively, over all data points required to complete the sample analysis.

In addition to the analysis of calibration standards specified in the protocol, solution HRCC6 was analyzed in triplicate to determine the lower limit of sensitivity (1 pg/μL). Although the calculated RRF(I) and RRF(II) values and the S/N are within the specified criteria, the ion ratio for the native compound and recovery standard indicate that these measurements fall outside the acceptable calibration window.

TABLE 10. INITIAL CALIBRATION SUMMARY

Calibration standard	Date	Time	m/z 320/322	m/z 332/334 (IS)	m/z 332/334 (RS)	S/N 259	S/N 322	S/N 334(IS)	RRF(I)	RRF(II)
HRCC1	09/12/85	09:05	0.82	0.77	0.80	41:1	> 65:1	> 65:1	0.783	2.18
HRCC1	09/12/85	09:44	0.84	0.80	0.73	48:1	> 65:1	> 65:1	0.794	2.27
HRCC1	09/12/85	12:31	0.84	0.73	0.87	24:1	> 65:1	> 65:1	0.750	2.28
								Mean	0.776	2.24
								% RSD	2.9%	2.4%
HRCC2	09/12/85	13:00	0.73	0.77	0.83	36:1	> 65:1	> 65:1	0.829	1.76
HRCC2	09/12/85	13:27	0.80	0.73	0.81	58:1	> 65:1	> 65:1	0.853	1.93
HRCC2	09/12/85	13:53	0.92	0.66	0.69	78:1	> 65:1	> 65:1	0.799[a]	2.03[a]
HRCC2	09/12/85	15:39	0.78	0.77	0.73	76:1	> 65:1	> 65:1	0.861	2.27
								Mean	0.848	1.99
								% RSD	2.0%	13.0%
HRCC3	09/13/85	10:31	0.78	0.79	0.79	97:1	> 65:1	> 65:1	0.974	1.53
HRCC3	09/13/85	10:57	0.78	0.80	0.78	111:1	> 65:1	> 65:1	0.965	1.57
HRCC3	09/13/85	11:23	0.73	0.83	0.78	110:1	> 65:1	> 65:1	0.972	1.58
								Mean	0.970	1.56
								% RSD	0.5%	1.9%
HRCC4	09/13/85	13:02	0.77	0.80	0.76	96:1	> 65:1	> 65:1	0.945	1.49
HRCC4	09/13/85	13:29	0.73	0.76	0.78	> 144:1	> 65:1	> 65:1	0.935	1.52
HRCC4	09/13/85	13:56	0.77	0.78	0.76	> 144:1	> 65:1	> 65:1	0.967	1.49
								Mean	0.949	1.50
								% RSD	1.7%	1.1%
HRCC5	09/13/85	14:22	0.78	0.80	0.78	> 144:1	> 65:1	> 65:1	0.964	1.52
HRCC5	09/13/85	14:49	0.75	0.82	0.83	> 144:1	> 65:1	> 65:1	1.01	1.42
HRCC5	09/13/85	15:15	0.76	0.78	0.79	> 144.1	> 65:1	> 65:1	0.989	1.43
								Mean	0.987	1.46
								% RSD	2.2%	3.9%
								Overall Mean (RRF)	0.906	1.75
								% RSD	9.4%	19.3%
HRCC6	09/16/85	10:43	1.32	0.71	0.84	10:1	21:1	> 65:1	0.917	1.44
HRCC6	09/16/85	11:19	1.18	0.83	1.14	9.6:1	25:1	> 65:1	0.878	1.09
HRCC6	09/16/85	13:44	0.86	0.80	1.01	18:1	30:1	> 65:1	0.935	1.58
HRCC1	09/16/85	12:42	0.87	0.83	0.75	36:1	63:1	> 65:1	0.876	1.86
HRCC1	09/16/85	14:40	0.83	0.79	0.85	48:1	> 63:1	> 65:1	0.850	2.11
HRCC2	09/20/85	10:46	0.86	0.73	0.80	> 75:1	> 63:1	> 63:1	0.835	1.98
HRCC2	09/23/85	08:47	0.82	0.80	0.70	> 75:1	> 63:1	> 63:1	0.832	2.65[b]
HRCC2	09/23/85	10:46	0.88	0.80	0.83	42:1	> 63:1	> 63:1	0.941	1.41
HRCC2	09/24/85	10:47	0.89	0.69	0.69	26:1	> 63:1	> 63:1	1.01	1.68
HRCC2	09/25/85	08:39	0.76	0.78	0.89	58:1	> 63:1	> 63:1	0.949	1.96
HRCC2	09/26/85	08:56	0.77	0.80	0.72	73:1	> 63:1	> 63:1	0.941	1.57
HRCC2	09/27/85	09:33	0.78	0.84	0.81	49:1	> 63:1	> 63:1	1.04	1.68
HRCC2	09/30/85	09:19	0.83	0.80	0.85	73:1	> 63:1	> 63:1	0.854	1.67
HRCC2	10/03/85	08:53	0.68	0.75	0.81	29:1	> 63:1	> 63:1	0.955	1.53

[a] Not included in mean RRF computation.
[b] Not within allowable limits for routine calibration.

TABLE 11. HRGC AND MASS RESOLUTION CHECK SUMMARY

Date	Inst. ID	Sol. ID	Time	File name	TCDD isomer resolution (% valley)	Mass resolution at 10% valley	Mass measurement error
9/12/85	MS50	-	07:51	MID254I12X1	-	10,774	5 ppm
9/12/85	MS50	PC	08:32	8367I12XQ1	5.9	-	-
9/12/85	MS50	PC	16:05	8367I12XQ9	2.9	-	-
9/12/85	MS50	-	16:47	MID254I12X2	-	10,450	-
9/13/85	MS50	-	08:26	MID254I13X1	-	10,230	0 ppm
9/13/85	MS50	PC	08:45	8367I13XQ1	6.9	-	-
9/13/85	MS50	PC	15:48	8367I13XQ12	11.4	-	-
9/13/85	MS50	-	16:23	MID254I13X2	-	10,384	-
9/16/85	MS50	-	09:25	MID254I16X1	-	10,294	4 ppm
9/16/85	MS50	PC	10:45	8367I16XQ1	11.9	-	-
9/16/85	MS50	PC	15:16	8367I16XQ8	23.0	-	-
9/16/85	MS50	-	15:57	MID254I16X2	-	10,388	-
9/17/85	MS50	PC	10:26	8367I17XQ1	13.3	-	-
9/17/85	MS50	-	10:14	MID254I17X1	-	10,824	2 ppm
9/18/85	MS50	-	07:58	MID254I18X1	-	11,019	1 ppm
9/18/85	MS50	PC	08:18	8367I18XQ1	20	-	-
9/19/85	MS50	-	11:14	MID254I19X1	-	11,679	4 ppm
9/19/85	MS50	PC	12:56	8367I19XQ1	3.5	-	-
9/20/85	MS50	-	08:00	MID254I20X1	-	12,068	3 ppm
9/20/85	MS50	PC	08:16	8367I20XQ1	6.7	-	-
9/20/85	MS50	PC	15:44	8367I20XQ5	4.1	-	-
9/20/85	MS50	-	16:14	MID254I20X3	-	10,777	-
9/23/85	MS50	-	07:55	MID254I23X1	-	10,096	1 ppm
9/23/85	MS50	PC	08:15	8367I23XQ1	8.8	-	-
9/23/85	MS50	PC	16:01	8367I23XQ6	12.5	-	-
9/23/85	MS50	-	16:45	Manual check[a]	-	12,500	-
9/24/85	MS50	-	09:51	MID254I24X1	-	10,374	3 ppm
9/24/85	MS50	PC	10:15	8367I24XQ1	12.2	-	-
9/24/85	MS50	PC	16:01	8367I24XQ3	13.1	-	-
9/24/85	MS50	-	16:33	MID254I24X2	-	10,567	-

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[a] A manual resolution check was performed due to data system failure.

(continued)

TABLE 11. (continued)

Date	Inst. ID	Sol. ID	Time	File name	TCDD isomer resolution (% valley)	Mass resolution at 10% valley	Mass measurement error
9/25/85	MS50	-	07:50	MID254I25X1	-	11,165	0 ppm
9/25/85	MS50	PC	08:05	8367I25XQ1	11.1	-	-
9/25/85	MS50	PC	16:13	8367I25XQ3	6.5	-	-
9/25/85	MS50	-	16:45	MID254I25X2	-	11,419	-
9/26/85	MS50	-	08:07	MID254I26X1	-	10,989	3 ppm
9/26/85	MS50	PC	08:21	8367I26XQ1	8.3	-	-
9/26/85	MS50	PC	15:49	8367I26XQ3	13.2	-	-
9/26/85	MS50	-	16:21	MID254I26X2	-	10,499	-
9/27/85	MS50	-	08:19	MID254I27X1	-	11,564	1 ppm
9/27/85	MS50	PC	09:02	8367I27XQ1	11.9	-	-
9/27/85	MS50	PC	16:01	8367I27XQ3	11.8	-	-
9/27/85	MS50	-	16:29	MID254I27X2	-	10,639	-
9/30/85	MS50	-	08:15	MID254I30X1	-	11,149	5 ppm
9/30/85	MS50	PC	08:33	8367I30XQ1	< 25	-	-
9/30/85	MS50	PC	15:10	8367I30XQ3	< 25	-	-
9/30/85	MS50	-	15:41	MID254I30X2	-	11,321	-
10/3/85	MS50	-	07:59	MID254J03X1	-	10,567	0 ppm
10/3/85	MS50	PC	08:20	8367J03XQ1	17	-	-
10/3/85	MS50	PC	15:56	8367J03XQ3	12	-	-
10/3/85	MS50	-	16:29	MID254J03X2	-	10,442	-

[a] A manual resolution check was performed due to data system failure.

TABLE 12. TCDD DATA REPORT FORM

							HRGC/HRMS Analysis			
Sample No.	Aliquot Air-dry wt. (g) or Vol. (L)	TCDD Isomer	Retention time TCDD	Retention time $^{13}C_{12}$-2,3,7,8	TCDD (ppt or ppq)[a] Meas.	DL	Instr. ID	Date	Time	Relati 320/322
8367-83-1576X-DWD	1.0 L	2,3,7,8-	21:35	21:34	228	-	MS50	09/20/85	11:27	0.78
		1,3,6,8-	16:43	-	117	-				0.69
		1,3,7,9-	17:54	-	86.5	-				0.80
8367-82-1576X-DW	1.0 L	2,3,7,8-	21:35	21:33	196	-	MS50	09/20/85	13:02	0.58
8367-89-1576X-EWWD	1.0 L	2,3,7,8-	21:40	21:37	2,277	-	MS50	09/20/85	14:41	0.79
		1,3,6,8-	16:48		134	-				1.02
		1,3,7,9-	17:58		282	-				0.84
		c	24:10		137	-				0.90
8367-88-1576X-EWW	1.0 L	2,3,7,8-	21:29	21:27	1,090	-	MS50	09/20/85	15:11	0.77
		c	23:57		75.9	-				0.89
8367-90-1576X-EWWN	1.0 L	2,3,7,8-	21:59	21:56	1,010	-	MS50	09/23/85	11:17	0.74
		1,3,6,8-	17:02		502	-				0.85
		1,3,7,9-	18:14		766	-				0.83
		1,2,3,7/ 1,2,3,8-	22:18		1,860	-				0.81
		1,2,3,4-	22:31		1,840	-				0.71
		1,2,7,8-	24:30		3,430	-				0.78
		1,2,8,9-	30:01		1,330	-				0.80
8367-85-1576X-IND	1.0 L	2,3,7,8-	21:55	21:55	1,290	-	MS50	09/23/85	12:51	0.81
8367-92-1576X-IWWD	1.0 L	2,3,7,8-	22:01	21:59	508	-	MS50	09/23/85	13:29	0.74
		1,3,6,8-	17:06		191	-				0.85
		1,3,7,9-	18:16		208	-				0.75
		1,2,7,8-	24:33		55.2	-				0.89
8367-87-1576X-INDN	1.0 L	2,3,7,8-	22:00	21:59	1,520	-	MS50	09/23/85	14:00	0.87
		1,2,7,8-	24:31		586	-				0.71
8367-84-1576X-DWN	1.0 L	2,3,7,8-	22:01	22:00	234	-	MS50	09/23/85	14:31	0.75
		1,3,6,8-	17:06		512	-				0.80
		1,3,7,9-	18:18		395	-				0.74
		1,2,3,7/ 1,2,3,8-	22:21		403	-				0.72
		1,2,3,4-	22:35		616	-				0.72
		1,2,7,8-	24:32		840	-				0.80
		1,2,8,9-	30:02		328	-				0.79

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[a] Aqueous sample data reported as ppq and soil sample data presented as ppt.

[b] Criteria for positive identification require that the ion ratios fall between 0.67 and 0.90.

[c] Isomer could not be identified.

(continued)

Analysis

Relative Ion Abundance Ratios[b]				S/N			
320/322	332/334(IS)	332/334(RS)	% Rec.	m/z 259	m/z 322	m/z 334(IS)	Comments
0.78	0.63	0.68	40	73:1	> 63:1	> 49:1	Ratio unacceptable. Rerun.
0.69	-	-		64:1	50:1	-	
0.80	-	-		41:1	30:1	-	
0.58	0.69	0.72	14	21:1	62:1	> 20:1	Ratio unacceptable; low recovery. Rerun.
0.79	0.72	0.71	96	> 146:1	> 63:1	> 63:1	Sample spiked at twice requested level. Rerun.
1.02				10:1	> 5:1		
0.84				28:1	> 11:1		
0.90				6:1	63:1		
0.77	0.71	0.72	61	146:1	> 63:1	> 63:1	
0.89				7:1	31:1		
0.74	0.72	0.82	91	27:1	34:1	> 63:1	
0.85				22:1	22:1		
0.83				30:1	33:1		
0.81				54:1	> 63:1		
0.71				45:1	60:1		
0.78				78:1	> 63:1		
0.80				22:1	18:1		
0.81	0.90	0.81	23	37:1	63:1	30:1	Low recovery.
0.74	0.80	0.71	75	49:1	63:1	> 63:1	
0.85				25:1	29:1		
0.75				27:1	29:1		
0.89				8:1	16:1		
0.87	0.78	0.86	20	49:1	63:1	23:1	Low recovery.
0.71				21:1	31:1		
0.75	0.71	0.74	82	23:1	-	> 63:1	
0.80				73:1	63:1		
0.74				53:1	50:1		
0.72				42:1	45:1		
0.72				61:1	55:1		
0.80				70:1	63:1		
0.79				23:1	20:1		

TABLE 12. (continued)

							HRGC/HRMS Analysis			
Sample No.	Aliquot Air-dry wt. (g) or Vol. (L)	TCDD Isomer	Retention time		TCDD (ppt or ppq)[a]		Instr. ID	Date	Time	Relati
			TCDD	$^{13}C_{12}$-2,3,7,8	Meas.	DL				320/322
8367-91-1576X-IWW	1.0 L	2,3,7,8-	22:00	21:57	534	-	MS50	09/23/85	15:01	0.87
		1,3,6,8-	17:03		246	-				0.71
		1,3,7,9-	18:14		222	-				0.78
		1,2,7,8-	24:30		54.7	-				0.70
8367-86-1576X-INDD	1.0 L	2,3,7,8-	21:50	21:48	1,430	-	MS50	09/23/85	15:31	0.80
8367-93-1576X-IWWN	1.0 L	2,3,7,8-	21:39	21:38	530	-	MS50	09/24/85	11:19	0.76
		1,3,6,8-	16:47		582	-				0.82
		1,3,7,9-	17:57		690	-				0.80
		1,2,3,7/ 1,2,3,8-	21:58		940	-				0.72
		1,2,3,4-	21:11		1,180	-				0.79
		1,2,7,8-	24:08		1,790	-				0.82
		1,2,8,9-	29:35		691	-				0.77
8367-70-1576X-H1N	10.01 g	2,3,7,8-	21:38	21:37	30.3	-	MS50	09/24/85	12:55	0.85
		1,3,6,8-	16:45		29.0	-				0.63
		1,3,7,9-	17:55		51.1	-				0.87
		1,2,3,7/ 1,2,3,8-	21:58		125	-				0.81
		1,2,3,4-	22:10		118	-				0.69
		1,2,7,8-	24:09		252	-				0.81
		1,2,8,9-	29:36		100	-				0.85
8367-65-1576X-B5	10.00 g	2,3,7,8-	21:47	21:45	18.2	-	MS50	09/24/85	13:27	0.87
		1,3,7,9-	18:05		8.5	-				0.85
8367-66-1576X-B5D	9.85 g	2,3,7,8-	21:44	21:43	15.1	-	MS50	09/24/85	13:58	0.67
		1,3,7,9-	18:03		4.2	-				0.87
8367-67-1576X-B5N	10.00 g	2,3,7,8-	21:47	21:46	12.9	-	MS50	09/24/85	14:29	0.86
		1,3,6,8-	16:55		ND	9.2				0.59
		1,3,7,9-	18:05		15.2	-				0.67
		1,2,3,7/ 1,2,3,8-	22:07		61.8	-				0.77
		1,2,3,4-	22:19		54.1	-				0.90
		1,2,7,8-	24:16		147	-				0.75
		1,2,8,9-	29:45		63.9	-				0.83

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[a] Aqueous sample data reported as ppq and soil sample data presented as ppt.
[b] Criteria for positive identification require that the ion ratios fall between 0.67 and 0.90.
[c] Isomer could not be identified.

(continued)

Analysis

Relative Ion Abundance Ratios[b]				S/N			
320/322	332/334(IS)	332/334(RS)	% Rec.	m/z 259	m/z 322	m/z 334(IS)	Comments
0.87	0.83	0.81	77	72:1	> 62:1	> 63:1	
0.71				47:1	48:1		
0.78				36:1	32:1		
0.70				7:1	16:1		
0.80	0.76	0.79	29	49:1	63:1	> 33:1	Low recovery.
0.76	0.72	0.73	71	23:1	49:1	> 63:1	
0.82				42:1	40:1		
0.80				43:1	41:1		
0.72				49:1	49:1		
0.79				50:1	64:1		
0.82				72:1	63:1		
0.77				22:1	20:1		
0.85	0.78	0.77	56	12:1	15:1	> 63:1	
0.63				15:1	26:1		
0.87				24:1	33:1		
0.81				46:1	63:1		
0.69				38:1	58:1		
0.81				73:1	> 63:1		
0.85				20:1	23:1		
0.87	0.78	0.75	73	12:1	42:1	> 63:1	
0.85				9.4:1	25:1		
0.67	0.80	0.72	85	18:1	31:1	> 63:1	
0.87				6.3:1	12:1		
0.86	0.73	0.71	48	9:1	16:1	> 63:1	
0.59				11:1	15:1		Ratio unacceptable.
0.67				14:1	20:1		
0.77				48:1	63:1		
0.90				35:1	58:1		
0.75				73:1	63:1		
0.83				26:1	22:1		

TABLE 12. (continued)

							HRGC/HRMS Analysis			
Sample No.	Aliquot Air-dry wt. (g) or Vol. (L)	TCDD Isomer	Retention time TCDD	Retention time $^{13}C_{12}$-2,3,7,8	TCDD (ppt or ppq)[a] Meas.	TCDD (ppt or ppq)[a] DL	Instr. ID	Date	Time	Relati 320/322
8367-68-1576X-H1	9.67 g	2,3,4,7-	21:42	21:40	34.3	-	MS50	09/24/85	15:02	0.82
		1,3,6,8-	16:48		4.5	-				0.67
8367-69-1576x-H1D	10.00 g	2,3,7,8-	21:33	21:31	36.6	-	MS50	09/24/85	15:32	0.70
		1,3,6,8-	16:42		5.2	-				0.69
		1,3,7,9-	17:52		10.0	-				0.88
8367-71-1576x-B1	1.02 g	2,3,7,8-	21:37	21:35	937	-	MS50	09/25/85	10:01	0.83
		1,3,6,8-	16:44		160	-				0.85
		1,3,7,9-	17:54		312	-				0.84
		c	19:04		50.6	-				0.69
8367-72-1576x-B1D	1.05 g	2,3,7,8-	21:35	21:33	785	-	MS50	09/25/85	10:30	0.78
		1,3,6,8-	16:42		201	-				0.87
		1,3,7,9-	17:53		308	-				0.82
		c	19:02		ND	28.9				0.65
8367-73-1576x-B1N	1.03 g	2,3,7,8-	21:41	21:41	1,280	-	MS50	09/25/85	11:27	0.77
		1,3,6,8-	16:48		333	-				0.77
		1,3,7,9-	17:59		635	-				0.85
		c	19:09		52	-				0.81
		1,2,3,6/ 1,2,3,8-	22:01		518	-				0.72
		1,2,3,4-	22:15		695	-				0.78
		1,2,7,8-	24:12		1,170	-				0.82
		1,2,8,9-	29:41		463	-				0.83
8367-74-1576x-H3	1.15 g	2,3,7,8-	21:44	21:42	2,020	-	MS50	09/25/85	13:00	0.81
		1,3,6,8-	16:49		164	-				0.86
		1,3,7,9-	18:00		237	-				0.81
		c	19:10		70.6	-				0.74
		1,2,7,8-	24:58		31.7	-				0.92
		c	26:03		27.3	-				0.68
8367-77-1576x-FA	9.94 g	2,3,7,8-	21:36	21:34	1,720	-	MS50	09/25/85	13:30	0.80
		1,3,6,8-	16:42		1,880	-				0.83
		1,3,7,9-	17:53		1,750	-				0.81
		c	19:02		1,250	-				0.80

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[a] Aqueous sample data reported as ppq and soil sample data presented as ppt.

[b] Criteria for positive identification require that the ion ratios fall between 0.67 and 0.90.

[c] Isomer could not be identified.

(continued)

Analysis

Relative Ion Abundance Ratios[b]				S/N			
320/322	332/334(IS)	332/334(RS)	% Rec.	m/z 259	m/z 322	m/z 334(IS)	Comments
0.82	0.67	0.75	73	29:1	63:1	> 63:1	
0.67				11:1	14:1		
0.70	0.74	0.81	46	39:1	31:1	> 54:1	
0.69				11:1	5:1		
0.88				15:1	10:1		
0.83	0.83	0.83	95	97:1	63:1	> 63:1	
0.85				34:1	14:1		
0.84				44:1	24:1		
0.69				7.3:1	4:1		
0.78	0.85	0.80	75	73:1	63:1	> 63:1	
0.87				36:1	22:1		
0.82				40:1	28:1		
0.65				6.5:1	3:1		Ratio unacceptable.
0.77	0.83	0.81	80	97:1	-	> 63:1	
0.77				47:1	23:1		
0.85				79:1	35:1		
0.81				6.7:1	4:1		
0.72				57:1	27:1		
0.78				51:1	29:1		
0.82				87:1	63:1		
0.83				23:1	29:1		
0.81	0.81	0.81	79	> 145:1	> 63:1	> 63:1	
0.86				22:1	> 6:1		
0.81				24:1	> 10:1		
0.74				9:1	> 3:1		
0.92				2.5:1	6:1		
0.68				3:1	6:1		
0.80	0.82	0.82	4	67:1	28:1	6:1	Low recovery.
0.83				109:1	38:1	-	
0.81				94:1	34:1	-	
0.80				60:1	22:1	-	

TABLE 12. (continued)

							HRGC/HRMS Analysis			
Sample No.	Aliquot Air-dry wt. (g) or Vol. (L)	TCDD Isomer	Retention time		TCDD (ppt or ppq)[a]		Instr. ID	Date	Time	Relativ
			TCDD	$^{13}C_{12}$-2,3,7,8	Meas.	DL				320/322
8367-77-1576X-FA (concluded)		c	20:00		1,220	-	MS50	09/25/85	13:30	0.84
		c	20.43		274	-				0.75
		c	21:25		109	-				0.77
		1,2,3,7/ 1,2,3,8-	21:54		675	-				0.80
		1,2,3,4-	22:08		4,640	-				0.81
		c	22:35		879	-				0.88
		1,2,7,8-	24:05		720	-				0.88
		c	24:49		3,460	-				0.80
		c	25:23		155	-				0.75
		c	25:55		3,430	-				0.78
		c	27:18		441	-				0.79
		c	28:07		2,920	-				0.68
		c	29:41		ND	58				0.57
8367-94-1576X-H2W	1.0 L	2,3,7,8-	21:42	21:41	NC	-	MS50	09/25/85	15:18	0.86
		1,3,6,8-	16:47		NC	-				0.81
		1,3,7,9-	17:58		NC	-				0.84
		c	19:08		NC	-				0.79
		c	20:06		NC	-				0.98
		c	20:49		NC	-				0.68
		1,2,7,8-	24:13		NC	-				0.75
		c	24:55		NC	-				0.80
		c	26:01		NC	-				0.72
		c	27:25		NC	-				0.82
		c	28:13		NC	-				0.81
8367-83-1576X-DWD	1.0 L	2,3,7,8-	21:11	21:10	265	-	MS50	09/26/85	09:56	0.79
		1,3,6,8-	16:25		ND	167				0.91
		1,3,7,9-	17:34		125	-				0.71
		c	23:39		50.7	-				0.71
8367-82-1576X-DW	1.0 L	2,3,7,8-	21:10	21:09	300	-	MS50	09/26/85	10:26	0.72
		1,3,6,8-	16:22		140	-				0.81
		1,3,7,9-	17:32		106	-				0.96

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[a] Aqueous sample data reported as ppq and soil sample data presented as ppt.

[b] Criteria for positive identification require that the ion ratios fall between 0.67 and 0.90.

[c] Isomer could not be identified.

(continued)

Analysis

Relative Ion Abundance Ratios[b]				S/N			
320/322	332/334(IS)	332/334(RS)	% Rec.	m/z 259	m/z 322	m/z 334(IS)	Comments
0.84	-	-	-	44:1	15:1	-	
0.75				16:1	5:1		
0.77				6:1	2.5:1		
0.80				32:1	11:1		
0.81				146:1	63:1		
0.88				31:1	14:1		
0.88				23:1	15:1		
0.80				80:1	56:1		
0.75				7:1	5:1		
0.78				86:1	63:1		
0.79				14:1	9:1		
0.68				68:1	46:1		
0.57				7:1	4:1		Ratio unacceptable.
0.86	8.3	0.82	ND	> 154:1	> 65:1	4:1	332/334(IS) Ratio unacceptable; no amount computations performed.
0.81				18:1	55:1	-	
0.84				12:1	40:1	-	
0.79				20:1	63:1	-	
0.98				6:1	20:1	-	
0.68				3:1	11:1	-	
0.75				3:1	3:1	-	
0.80				37:1	35:1	-	
0.72				63:1	63:1	-	
0.82				9:1	8:1	-	
0.81				30:1	25:1	-	
0.79	0.73	0.70	42	18:1	31:1	47:1	
0.91				18:1	24:1	-	Ratio unacceptable.
0.71				14:1	21:1	-	
0.71				3.2:1	7:1	-	
0.72	0.70	0.74	16	10:1	21:1	22:1	Low recovery. Rerun.
0.81				6.3:1	12:1	-	
0.96				3.8:1	11:1	-	

TABLE 12. (continued)

Sample No.	Aliquot Air-dry wt. (g) or Vol. (L)	TCDD Isomer	Retention time		TCDD (ppt or ppq)[a]		HRGC/HRMS Analysis			
			TCDD	$^{13}C_{12}$-2,3,7,8	Meas.	DL	Instr. ID	Date	Time	Relati 320/322
8367-88-1576X-EWW	1.0 L	2,3,7,8-	21:18	21:15	1,030	-	MS50	09/26/85	12:32	0.76
		1,3,6,8-	16:28		119	-				0.71
		1,3,7,9-	17:38		221	-				0.68
		1,2,7,8-	23:44		119	-				0.76
8367-95-1576X-H2WD	1.0 L	2,3,7,8-	21:13	21:12	NC	-	MS50	09/26/85	13:05	0.93
		1,3,6,8-	16:25		NC	-				0.77
		1,3,7,9-	17:35		NC	-				0.82
		c	18:43		NC	-				0.81
		c	19:38		NC	-				0.87
		c	20:20		NC	-				0.79
		c	22:11		NC	-				0.84
		1,2,7,8-	23:39		NC	-				0.70
		c	24:23		NC	-				0.76
		c	25:27		NC	-				0.76
		c	26:47		NC	-				0.79
		c	27:37		NC	-				0.73
		c	29:34		NC	-				0.71
		c	30:12		NC	-				0.81
		c	31:07		NC	-				0.58
8367-96-1576X-H2WN	1.0 L	2,3,7,8-	21:14	21:13	NC	-	MS50	09/26/85	14:21	0.87
		1,3,6,8-	16:26		NC	-				0.79
		1,3,7,9-	17:35		NC	-				0.78
		c	18:44		NC	-				0.78
		c	19:41		NC	-				0.89
		c	20:22		NC	-				0.63
		c	21:13		NC	-				0.59
		1,2,7,8-	23:40		NC	-				0.79
		c	24:24		NC	-				0.73
		c	25:30		NC	-				0.75
		c	26:51		NC	-				0.76
		c	27:39		NC	-				0.70
		c	29:36		NC	-				0.77
		c	30:14		NC	-				0.74
		c	31:12		NC	-				0.33

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[a] Aqueous sample data reported as ppq and soil sample data presented as ppt.

[b] Criteria for positive identification require that the ion ratios fall between 0.67 and 0.90.

[c] Isomer could not be identified.

(continued)

Analysis

Relative Ion Abundance Ratios[b]				S/N			
320/322	332/334(IS)	332/334(RS)	% Rec.	m/z 259	m/z 322	m/z 334(IS)	Comments
0.76	0.79	0.75	66	73:1	> 63:1	> 63:1	
0.71				12:1	> 10:1	-	
0.68				17:1	> 15:1	-	
0.76				6:1	31:1	-	
0.93	13.3	0.67	NC	> 146:1	> 63:1	32:1	332/334(IS) Ratio unacceptable; no amount computations performed.
0.77				76:1	> 26:1	-	
0.82				42:1	> 14:1	-	
0.81				46:1	> 17:1	-	
0.87				15:1	> 5:1	-	
0.79				9:1	> 4:1	-	
0.84				5:1	> 2:1	-	
0.70				5:1	3.5:1	-	
0.76				54:1	39:1	-	
0.76				79:1	63:1	-	
0.79				11:1	8:1	-	
0.73				36:1	25:1	-	
0.71				7:1	5:1	-	
0.81				20:1	15:1	-	
0.58				4:1	2.5:1	-	
0.87	9.47	0.87	NC	> 145:1	> 63:1	3:1	332/334(IS) Ratio unacceptable; no amount computations performed.
0.79				40:1	> 23:1	-	
0.78				22:1	> 15:1	-	
0.78				34:1	> 21:1	-	
0.89				11:1	> 6:1	-	
0.63				7:1	> 5:1	-	Ratio unacceptable.
0.59				4:1	> 6:1	-	Ratio unacceptable.
0.79				5:1	> 3:1	-	
0.73				58:1	> 41:1	-	
0.75				89:1	> 69:1	-	
0.76				11:1	> 10:1	-	
0.70				39:1	> 28:1	-	
0.77				9:1	> 6:1	-	
0.74				23:1	> 20:1	-	
0.33				3:1	> 25:1	-	Ratio unacceptable.

TABLE 12. (continued)

Sample No.	Aliquot Air-dry wt. (g) or Vol. (L)	TCDD Isomer	Retention time		TCDD (ppt or ppq)[a]		HRGC/HRMS Analysis			
			TCDD	$^{13}C_{12}$-2,3,7,8	Meas.	DL	Instr. ID	Date	Time	Relativ 320/322
8367-75-1576X-H3D	1.16 g	2,3,7,8-	21:20	21:19	2,260	-	MS50	09/27/85	15:31	0.79
		1,3,6,8-	16:31		116	-				0.90
		1,3,7,9-	17:41		163	-				0.67
		c	18:49							
8367-76-1576-X-H3N	1.14 g	2,3,7,8-	21:39	21:39	1,800	-	MS50	09/30/85	09:57	0.80
		1,3,6,8-	16:45		383	-				0.80
		1,3,7,9-	17:56		367	-				0.81
		c	19:07		ND	49.5				1.00
		1,2,3,7/ 1,2,3,8-	21:58		825	-				0.81
		1,2,3,4-	22:11		855	-				0.83
		1,2,7,8-	24:10		2,330	-				0.85
		1,2,8,9-	29:36		952	-				0.77
8367-78-1576X-FAD	10.04 g	2,3,7,8-	21:33	21:30	1,020	-	MS50	09/30/85	10:29	0.73
		1,3,6,8-	16:41		926	-				0.77
		1,3,7,9-	17:51	-	747	-				0.80
		c	19:01		610	-				0.87
		c	19:57		557	-				0.89
		c	20:41		146	-				0.71
		1,2,3,7/ 1,2,3,8-	21:52		286	-				0.67
		1,2,3,4-	22:05		2,260	-				0.79
		c	22:33		ND	329				0.91
		c	24:01		356	-				0.74
		c	24:45		3,520	-				0.78
		c	25:51		3,680	-				0.79
		c	27:14		558	-				0.83
		c	28:03		3,120	-				0.76
		c	30:01		3,270	-				0.79
8367-99-1576X-FAN	9.93 g	2,3,7,8-	21:39	21:38	1,160	-	MS50	09/30/85	13:00	0.80
		1,3,6,8-	16:45		1,390	-				0.78
		1,3,7,9-	17:56		1,160	-				0.79
		c	19:06		881	-				0.85
		c	20:01		888	-				0.74
		c	20:47		194	-				0.76
		1,2,3,7/ 1,2,3,8-	21:58		423	-				0.80
		1,2,3,4-	22:11		3,620	-				0.86

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[a] Aqueous sample data reported as ppq and soil sample data presented as ppt.

[b] Criteria for positive identification require that the ion ratios fall between 0.67 and 0.90.

[c] Isomer could not be identified.

(continued)

Analysis

Relative Ion Abundance Ratios[b]				S/N			
320/322	332/334(IS)	332/334(RS)	% Rec.	m/z 259	m/z 322	m/z 334(IS)	Comments
0.79	0.88	0.80	99	> 145:1	> 63:1	> 63:1	
0.90				14:1	> 5:1		
0.67				20:1	> 7:1		
				6:1	> 2.5:1		
0.80	0.79	0.80	86	> 143:1	> 63:1	> 63:1	
0.80				44:1	> 15:1		
0.81				41:1	> 17:1		
1.00				5:1	> 2.5:1		Ratio unacceptable.
0.81				79:1	> 29:1		
0.83				60:1	> 24:1		
0.85				145:1	63:1		
0.77				46:1	23:1		
0.73	0.71	0.88	6.7	45:1	32:1	9:1	Recovery low.
0.77				55:1	37:1		
0.80				42:1	25:1		
0.87				32:1	22:1		
0.89				23:1	14:1		
0.71				8.7:1	6:1		
0.67				17:1	10:1		
0.79				75:1	63:1		
0.91				16:1	11:1		Ratio unacceptable.
0.74				13:1	8:1		
0.78				92:1	56:1		
0.79				97:1	63:1		
0.83				17:1	10:1		
0.76				80:1	48:1		
0.79				83:1	47:1		
0.80	0.84	0.88	5	56:1	25:1	6:1	Low recovery.
0.78				94:1	40:1		
0.79				80:1	28:1		
0.85				60:1	18:1		
0.74				35:1	17:1		
0.76				13:1	5:1		
0.80				29:1	10:1		
				131:1	63:1		
0.86				32:1	10:1		

TABLE 12. (concluded)

									HRGC/HRMS Analysis	
Sample No.	Aliquot Air-dry wt. (g) or Vol. (L)	TCDD Isomer	Retention time		TCDD (ppt or ppq)[a]		Instr. ID	Date	Time	Relati
			TCDD	$^{13}C_{12}$-2,3,7,8	Meas.	DL				320/322
8367-99-1576X-FAN (concluded)		c	22:38		562	-	MS50	09/30/85	13:00	0.81
		1,2,7,8-	24:08		516	-				0.82
		c	24:52		4,310	-				0.75
		c	25:58		4,530	-				0.73
		c	27:20		ND	632				0.95
		c	28:10		3,980	-				0.77
		c	30:09		4,080	-				0.88
		c	30:47		1,170	-				0.71
8367-100-1576X-DWD	1.0 L	2,3,7,8-	21:04	21:01	246	-	MS50	10/03/85	11:32	0.86
		1,3,6,8-	16:19		637	-				0.77
		1,3,7,9-	17:26		489	-				0.80
8367-102-1576X-IND	500 mL	2,3,7,8-	21:02	21:03	604	-	MS50	10/03/85	12:58	0.71
8367-101-1576X-EWWD	1.0 mL	2,3,7,8-	20:58	20:55	1,050	-	MS50	10/03/85	13:28	0.69
		1,3,6,8-	16:14		157					0.81
		1,3,7,9-	17:23		384					
8367-103-1576X-IND	500 mL	2,3,7,8-	20:57	20:56	628	-	MS50	10/03/85	14:01	0.71
		1,3,6,8-	16:17		ND	45				0.52
		1,3,7,9-	17:23		87	-				0.69
8367-104-1576X-H2W	430 mL	2,3,7,8-	21:00	20:59	27,100	-	MS50	10/03/85	14:33	0.74
		1,3,6,8-	16:18		ND	71				1.05
		1,3,7,9-	17:27		164	-				0.68
		c	18:32		391	-				0.85
		c	24:06		ND	427				0.65
		c	25:09		531	-				0.70
		c	27:16		575	-				0.81
		c	29:49		313	-				0.86
8367-105-1576X-H2W	430 mL	2,3,7,8-	20:56	20:56	28,100	-	MS50	10/03/85	15:17	0.80
		1,3,6,8-	16:15		224	-				0.85
		1,3,7,9-	17:22		302	-				0.88
		c	18:30		453	-				0.73
		c	24:03		564	-				0.80
		c	25:07		730	-				0.81
		c	24:17		518	-				0.80
		c	29:45		347	-				0.79

[a] Aqueous sample data reported as ppq and soil sample data presented as ppt.
[b] Criteria for positive identification require that the ion ratios fall between 0.67 and 0.90.
[c] Isomer could not be identified.

Analysis

Relative Ion Abundance Ratios[b]				S/N			
320/322	332/334(IS)	332/334(RS)	% Rec.	m/z 259	m/z 322	m/z 334(IS)	Comments
0.81	-	-	5	32:1	10:1		
0.82				20:1	10:1		
0.75				127:1	54:1		
0.73				146:1	63:1		
0.95				20:1	7:1		Ratio unacceptable.
0.77				24:1	46:1		
0.88				122:1	44:1		
0.71				26:1	14:1		
0.86	0.82	0.74	68.5	21:1	18:1	> 63:1	
0.77				73:1	63:1		
0.80				51:1	47:1		
0.71	0.87	0.85	59.5	16:1	31:1	> 63:1	
0.69	0.82	0.90	80	72:1	63:1	> 63:1	
0.81				17:1	13:1		
				28:1	26:1		
0.71	0.75	0.78	57	18:1	42:1	> 63:1	
0.52				6:1	13:1		
0.69				8:1	17:1		
0.74	0.84	0.74	78	> 145:1	> 63:1	> 63:1	
1.05				9:1	18:1		Ratio unacceptable.
0.68				16:1	31:1		
0.85				30:1	55:1		
0.65				19:1	21:1		Ratio unacceptable.
0.70				32:1	32:1		
0.81				28:1	26:1		
0.86				18:1	27:1		
0.80	0.84	0.71	96	> 145:1	> 63:1	> 63:1	
0.85				16:1	19:1		
0.88				17:1	21:1		
0.73				30:1	34:1		
0.80				20:1	14:1		
0.81				33:1	31:1		
0.80				23:1	14:1		
0.79				13:1	10:1		

TABLE 13. ACCURACY AND PRECISION OF THE HRGC/HRMS ANALYSIS FOR 2,3,7,8-TCDD FROM LABORATORY AQUEOUS MATRIX SPIKES

Sample matrix	2,3,7,8-TCDD Spike level (ppq)	2,3,7,8-TCDD Detected (ppq)	2,3,7,8-TCDD Recovery (%)	$^{13}C_{12}$-2,3,7,8-TCDD Absolute recovery (%)
Distilled water (DW)	250	234	93.6	82
	250	265	106	42
	250	246	103	69
		Average conc. 248	Average rec. 101	Average rec. 64
		RPR[a] 12.5	RPR 9.3	RPR 63
Effluent wastewater (EWW)	1,000	1,090, 1,030	109, 103	61, 66
	1,000	1,010	101	91
	1,000	1,050	105	80
		Average conc. 1,050	Average rec. 105	Average rec. 75
		RPR 7.6	RPR 7.6	RPR 40
Influent wastewater (IWW)	500	534	107	77
	500	508	102	75
	500	530	106	71
		Average conc. 524	Average rec. 105	Average rec. 74
		RPR 5.0	RPR 4.8	RPR 8.1
Industrial wastewater (IND)	500	1,290	258	23
	500	1,520	304	20
	500	1,430	286	29
		Average conc. 1,410	Average rec. 283	Average rec. 24
		RPR 16	RPD 16	RPR 38
Industrial wastewater (IND)	-	604	-	60
	-	628	-	57
		Average conc. 616		Average rec. 58
		RPD[b] 3.9		RPD 5.2
Soil extract (H2W)	-	27,100	-	78
	-	28,100	-	96
		Average conc. 27,600		Average rec. 87
		RPD 3.6		RPD 25

[a] Relative percent range (calculated from the difference of the high and low values divided by the average of all values and multiplied by 100 percent).

[b] Relative percent difference.

TABLE 14. PRECISION OF THE HRGC/HRMS ANALYSIS FOR 2,3,7,8-TCDD OF SOIL AND FLY ASH SAMPLES

Sample matrix	Endogenous 2,3,7,8-TCDD level (ppt)[a]		2,3,7,8-TCDD Detected (ppt)		$^{13}C_{12}$-2,3,7,8-TCDD Absolute recovery (%)
B25-Piazza Road (B5)	50		18.2		73
			15.1		85
			12.9		48
		Average conc.	15.4	Average rec.	69
		RPR[b]	34	RPR	54
Hyde Park 001 (H1)	70		34.3		73
			36.6		46
			30.3		56
		Average conc.	33.7	Average rec.	58
		RPR	19	RPR	47
B52-Shenandoah (B1)	360		937		95
			785		75
			1,280		80
		Average conc.	1,000	Average rec.	83
		RPR	50	RPR	24
Hyde Park 003 (H3)	1,700		2,020		79
			2,260		99
			1,800		86
		Average conc.	2,030	Average rec.	88
		RPR	23	RPR	23
Fly ash - RRAI	-		1,720		4
			1,020		7
			1,160		5
		Average conc.	1,300	Average rec.	5.3
		RPR	54	RPR	57

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[a] Estimated level of endogenous 2,3,7,8-TCDD reported to MRI by Dr. W. Beckert in letters dated April 19, 1985, and August 30, 1985.

[b] Relative percent range (calculated from the difference of the high and low values, divided by the average of all values, and multiplied by 100 percent.

TABLE 15. ACCURACY OF THE HRGC/HRMS METHOD FOR THE DETERMINATION OF TCDD ISOMERS SPIKED INTO AQUEOUS MATRICES

TCDD analyte	Effluent wastewater			Distilled water			Influent wastewater			Industrial wastewater		
	Spike (pg)	Measured (pg)	Recovery (%)	Spike (pg)	Measured (pg)	Recovery (%)	Spike (pg)	Measured (pg)	Recovery (%)	Spike (pg)	Measured (pg)	Recovery (%)
1,3,6,8	1,840	502	27	460	512	111	920	582	63	920	ND[a]	0
1,3,7,9	840	766	91	210	395	190	420	690	164	420	ND	0
1,2,3,7/1,2,3,8	1,680	1,860	110	420	403	96	840	940	112	840	ND	0
1,2,3,4	2,440	1,840	75	610	616	101	1,220	1,180	97	1,220	ND	0
1,2,7,8	3,080	3,430	111	770	840	110	1,540	1,790	116	1,540	586	38
1,2,8,9	1,200	1,330	111	300	328	110	600	691	115	600	ND	0
2,3,7,8	1,000	1,010	101	250	234	94	500	530	106	500	904[b]	181
$^{13}C_{12}$-2,3,7,8	500	455	91	500	410	82	500	355	71	500	100	20

[a] Not detected.
[b] Measured value corrected for endogenous 2,3,7,8-TCDD content (averaged 616 pg/L).

TABLE 16. ACCURACY OF THE HRGC/HRMS METHOD FOR THE DETERMINATION OF TCDD ISOMERS SPIKED INTO SOIL MATRICES

TCDD analyte	Hyde Park 001 (H1)			B25-Piazza Road (B5)			B52-Shenandoah (B1)			Hyde Park 003 (H3)		
	Spike (pg)	Measured (pg)	Recovery (%)	Spike (pg)	Measured (pg)	Recovery (%)	Spike (pg)	Measured (pg)	Recovery (%)	Spike (pg)	Measured (pg)	Recovery (%)
1,3,6,8	130	29.0	22	92	ND (9.2)[a]	0	660	333	50	1,560	383	24
1,3,7,9	60	51.1	86	42	15.2	36	300	635	210	710	367	51
1,2,3,7/1,2,3,8	120	125	106	84	61.8	74	600	518	87	1,430	825	58
1,2,3,4	170	118	69	120	54.1	44	880	695	79	2,070	855	41
1,2,7,8	220	252	117	150	147	95	1,110	1,170	106	2,620	2,330	89
1,2,8,9	84	100	119	60	63.9	107	430	463	108	1,020	952	93
2,3,7,8	-	30.3	-	-	12.9	-	-	1,280	-	-	1,800	-
$^{13}C_{12}$-2,3,7,8	500	280	56	500	240	48	500	400	80	500	430	86

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[a] ND - not detected. The value in parentheses reflects the estimated detection limit.

TABLE 17. FORTIFIED FIELD BLANK RESULTS

Sample No.	Aliquot Air-dry wt. (g) or Vol. (L)	Retention time		Instr. ID	Date	Time	Relati
		Native	^{13}C				320/322
8367-62-1576X-FFWB	1.0 L	23:38	23:35	MS50	09/11/85	14:08	0.78
8367-64-1576X-FFSB	10 g	23:38	23:38	MS50	09/11/85	14:42	0.79
8367-63-1576X-FFSA	10 g	23:40	23:38	MS50	09/12/85	14:47	0.77
8367-61-1576X-FFWA	1.0 L	23:40	23:39	MS50	09/12/85	15:14	0.80
8367-81-1576X-FFB	10.01 g	22:17	22:15	MS50	09/20/85	13:33	0.88
8367-80-1576X-FFA	10.01 g	21:37	21:35	MS50	09/20/85	14:09	0.86
8367-97-1576X-FFA	1.0 L	21:37	21:37	MS50	09/20/85	09:10	0.82
8367-98-1576X-FFB	1.0 L	21:43	21:43	MS50	09/20/85	09:24	0.80

Relative Ion Abundance Ratios				S/N			
320/322	332/334(IS)	332/334(RS)	% Rec.	m/z 259	m/z 322	m/z 334(IS)	Comments
0.78	-	0.71	65	> 145:1	> 63:1	> 63:1	
0.79	-	0.80	68	> 145:1	> 63:1	> 63:1	
0.77	0.78	-	71	145:1	> 63:1	> 63:1	
0.80	0.76	-	79	145:1	> 63:1	> 63:1	
0.88	-	0.74	29	144:1	> 63:1	> 63:1	Low recovery
0.86	0.73	-	83	145:1	> 63:1	> 63:1	
0.82	0.83	-	50	23:1	> 63:1	> 63:1	
0.80	-	0.82	48	145:1	> 63:1	> 63:1	

HRGC and Mass Resolution

Table 11 presents a summary of all chromatographic and mass resolution checks completed during the final method evaluation. As per the protocol requirements the required mass resolution was demonstrated as the first and last quality control requirements for each day. The column performance check mixture was also analyzed before the first sample analysis and after the final sample analysis each day as a QC measure to assure that specificity for 2,3,7,8-TCDD was maintained. The mass measurement accuracy at m/z 330.979 is also included in this table, as it was verified on a daily basis prior to any sample analyses.

Sample Analysis

The results from the analysis of the aqueous and soil samples are provided in Table 12. The data in Table 12 are presented in the format specified as Form B-1 in the protocol reporting requirement. The data are recorded in the chronological order that they were obtained by HRGC/HRMS.

As indicated in Table 12, several samples required reanalysis due to low recovery of the internal standard, unacceptable ion ratios for 320/322, or the result of interferences at the internal standard. Two of the distilled water samples demonstrated responses for the characteristic ions at m/z 259, 320, and 322 for 2,3,7,8-TCDD. However, the ion ratio for the native 2,3,7,8-TCDD in one replicate and the ion ratio for the internal standard in another required that both samples be reanalyzed. Although both samples met all the qualitative criteria, recoveries were noted to be low (< 20 percent) for one of the samples and complete reanalysis of the replicate was required.

Significant problems were encountered with the aqueous soil extract, H2W, and the fly ash sample. The problems with the soil extract resulted from an interference at m/z 332 that coeluted with the internal standard, $^{13}C_{12}$-2,3,7,8-TCDD. No accurate quantitative measurements could be achieved for TCDD responses observed for this sample. The original sample contained a large amount of suspended particulate in each of the three replicates. Problems with the extraction were noted with the first portion of methylene chloride. A large amount of particulate matter was noted at the interface of the aqueous and organic phases. Hence, the interference at m/z 332 and TCDD responses observed in these replicates were probably due to direct extraction of the suspended soil particulate rather than the actual water-soluble TCDD.

The remaining aqueous sample for H2W was centrifuged for 10 min at approximately 2,000 rpm, and the aqueous phase was decanted from the settled particulate. The resulting aqueous sample was divided into duplicate 430-mL samples and each was analyzed according to the protocol. The HRGC/HRMS analysis was successful for both replicates with absolute recoveries of 78 percent and 96 percent of the internal standard.

The triplicate analyses of the fly ash sample resulted in absolute recoveries less than 10 percent for the internal standard in each aliquot analyzed. These low recoveries may be associated with the total fixed carbon content of the fly ash material. Previous work in this laboratory with fly ash from coal-fired power plants has demonstrated low recoveries of analytes from materials with high carbon content.[4]

The only other sample for which successful analysis was not achieved as specified in the protocol on first analysis was the industrial wastewater (IND). The triplicate analysis of the sample resulted in absolute internal standard recoveries of 23, 20, and 29 percent. The criteria for successful analysis for TCDD as discussed in the protocol require an absolute recovery of 40 to 120 percent. In addition to the observed low recoveries, the level of 2,3,7,8-TCDD detected in the sample averaged 1,410 ppq as compared to the 500-ppq spike level. Two 500-mL aliquots of the unspiked industrial wastewater sample were reanalyzed to determine the background level of 2,3,7,8-TCDD. The results of the duplicate analysis yielded an average 2,3,7,8-TCDD concentration of approximately 620 ppq and the absolute recoveries were noted to be 60 percent and 57 percent. The increase in absolute recovery of the internal standard in the unspiked sample by approximately a factor of two is possibly due to the preparation of samples one half the size of that used for the original analysis. This suggests that the sample matrix has a considerable impact on the effectiveness of the cleanup procedure.

Table 13 provides a summary of the accuracy and precision of the analyses of the five aqueous sample types for 2,3,7,8-TCDD. Only the data points from Table 12 that demonstrate compliance with all QC criteria (ion ratios, absolute recovery of the internal standard, etc.) are included in Table 13. These data demonstrate that the isotope dilution method of quantitation provides accurate and precise quantitation of 2,3,7,8-TCDD in the aqueous samples. It should be noted that even when the absolute recovery of the $^{13}C_{12}$-2,3,7,8-TCDD internal standard varies by as much as 66 percent (RPR) for the triplicate distilled water samples, the accuracy of the measurement of the spiked 2,3,7,8-TCDD averaged 101 percent with less than 10 percent variability. Table 13 summarizes data for both the spiked and unspiked aliquots of industrial wastewater. The high recovery noted for the 2,3,7,8-TCDD value in the spiked samples is a result of the presence of this compound at approximately 620 ppq in the original matrix.

Table 14 presents a similar summary for the five solid samples analyzed. The precision of the measurements is not quite as good as noted for the aqueous samples and may reflect the difference in adsorption of the endogenous 2,3,7,8-TCDD and the spiked internal standard to the matrices.

Tables 15 and 16 provide data dealing with the accuracy of the HRGC/HRMS methods for the determination of total TCDD isomers in aqueous and solid samples. In general, the data support the use of the internal standard method of quantitation for all but the earliest eluting isomers, 1,3,6,8- and 1,3,7,9-TCDD. The accuracy for the additional isomers is very good and more consistent than is observed for the solid samples. This may be partially due to the differences in adsorption to the soil particles.

Fortified Field Blanks

As part of the overall quality assurance/quality control (QA/QC) program identified in the HRGC/HRMS protocol, the analyst is required to analyze fortified field blanks to demonstrate (a) that the extraction and cleanup procedure will provide recovery of the 2,3,7,8-TCDD within the criteria of greater than 40 percent specified in the protocol and (b) that the reagents are free from contamination with TCDD isomers.

Table 17 provides the results of the fortified field blanks run before proceeding with sample analysis and also those of an additional set of blanks prepared along with the actual samples. The analyses of the fortified field blanks at the outset of the study demonstrated that the recoveries of 2,3,7,8-TCDD and 1,2,3,4-TCDD ranged from 65 to 79 percent. No detectable levels of other TCDD isomers were found in this preliminary study. The field fortification blanks analyzed with the actual samples resulted in recoveries of 29 percent and 83 percent. More importantly, these analyses demonstrated some interferences arising from 1,3,6,8- and 1,3,7,9-TCDD. Previous studies involving evaluation of the cleanup procedure indicated that these isomers are associated with the acidic alumina cleanup.

Figure 4 is a plot of the ratio of response of 1,3,6,8- and 1,3,7,9-TCDD and the response of the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD versus the time elapsed since the acidic alumina was cleaned and activated at 190°C. The results of the analyses of the fortified field blanks and the samples not spiked with the 1,3,6,8- and 1,3,7,9-TCDD isomers are presented in Figure 4. As noted from this plot, these TCDD isomers were not initially detected in the acidic alumina immediately following cleanup by Soxhlet extraction. The first set of fortified field blanks was taken through the acidic alumina column 7 days later. Although response was observed at m/z 320 and 322 at the retention time for these isomers, the ion ratios did not indicate presence of the compounds. Since the detectable levels were well below 10 pg/g of alumina, the sample analyses were initiated. The data for the fortified field blanks and samples taken through alumina from 14 to 30 days from activation indicate that the contamination of the 1,3,6,8- and 1,3,7,9-TCDD isomers apparently occurs over time using this particular oven. The background contamination of 1,3,6,8- and 1,3,7,9-TCDD isomers has also been recently addressed by the Center for Disease Control.[5]

Note Added in Proof

A second magnetic sector instrument (built in 1976) from a different manufacturer was tested and found to be incapable of achieving sufficient sensitivity at 10,000 resolving power to be used in experiments for this study.

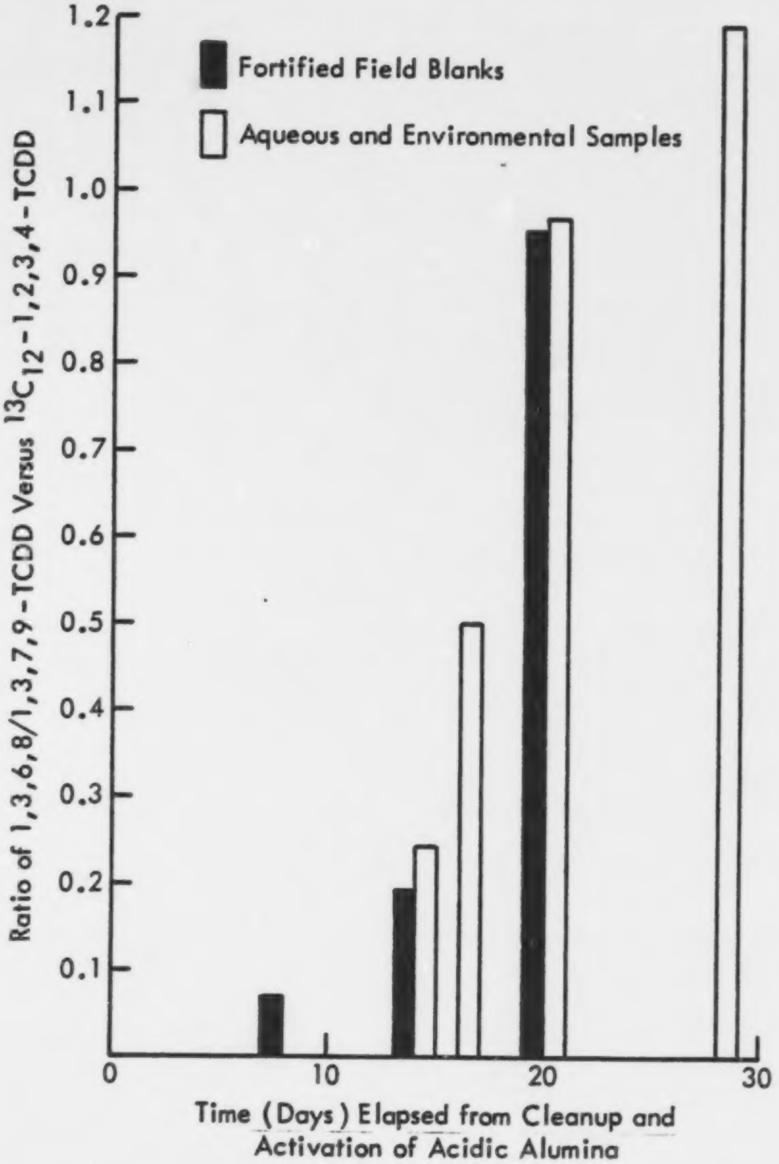

Figure 3. Background levels of 1,3,6,8- and 1,3,7,9-TCDD observed over the single-laboratory evaluation study.

REFERENCES

1. U.S. Environmental Protection Agency, "Dioxin Strategy," prepared by the Office of Water Regulations and Standards and the Office of Solid Waste and Emergency Response in conjunction with the Dioxin Strategy Task Force, Washington, D.C., November 28, 1983.

2. L. R. Williams, Validation of Testing/Measurement Methods. EPA 600/X-83-060, 1983.

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4. C. L. Haile, J. S. Stanley, T. Walker, G. R. Cobb, and B. A. Boomer, "Comprehensive Assessment of the Specific Compounds Present in Combustion Processes. Volume 3. National Survey of Organic Emissions from Coal-Fired Utility Boiler Plants," EPA-560/5-83-006, September 1983.

5. J. S. Heller, D. G. Patterson, L. R. Alexander, D. F. Groce, R. P. O'Connor, and C. R. Lapeza, "Control of Artifacts and Contamination in the Development of a Dioxin Analytical Program," presented at the 33rd Annual Conference on Mass Spectrometry and Allied Topics, May 26-31, 1985, San Diego, California.

APPENDICES

CS-108-85
GAM
11-27-85

APPENDIX A

VALIDATED ANALYTICAL PROTOCOL

for the Determination of
2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and Total
TCDDs in Soil/Sediment and Water by High-Resolution Gas
Chromatography/High-Resolution Mass Spectrometry

September 10, 1985

This analytical protocol has been written in the format used in the Superfund program, as "Exhibit D" of a Statement of Work which in turn is part of an Invitation-for-Bid package under the Superfund Contract Laboratory Program. The other exhibits of the Statement of Work, although cited in Exhibit D, do not pertain to this method evaluation study.

EXHIBIT D

Analytical Methods

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and Total TCDDs in Soil/Sediment and Water by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry

EXHIBIT D

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1. SCOPE AND APPLICATION

1.1 This method provides procedures for the detection and quantitative measurement of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD; CAS Registry Number 1746-01-6; Storet number 3475) at concentrations of 10 pg/g (10 parts per trillion) to 200 pg/g (200 parts per trillion) in 10-g portions of soil and sediment and at 100 pg/L (100 parts per quadrillion) to 2000 pg/L (2 parts per trillion) in 1-L samples of water. The use of 1-g or 100-mL portions permits measurements of concentrations up to 2,000 pg/g (2 parts per billion) or 20 ng/L, respectively. This method also allows the estimation of quantities of total TCDD present in the sample. Samples containing concentrations of 2,3,7,8-TCDD greater than 2 ppb or 20 ng/L must be analyzed by a protocol designed for such concentration levels, with an appropriate instrument calibration range.

1.2 The minimum measurable concentration is estimated to be 10 pg/g (10 parts per trillion) for soil and sediment samples and 100 pg/L for water samples, but this depends on kinds and concentrations of interfering compounds in the sample matrix.

1.3 This method is designed for use by analysts who are experienced in the use of high-resolution gas chromatography/high-resolution mass spectrometry.

CAUTION: TCDDs are extremely hazardous. It is the laboratory's responsibility to ensure that safe handling procedures are employed.

2. SUMMARY OF METHOD

Five hundred pg of $^{13}C_{12}$-2,3,7,8-TCDD (internal standard) are added to a 10-g portion of a soil/sediment sample (weighed to 3 significant figures) or a 1-L aqueous sample and the sample is extracted with 200 to 250 mL benzene using a Soxhlet apparatus with a minimum of 3 cycles per hour or a continuous liquid-liquid extractor for 24 hours. A separatory funnel and 3 x 60 mL methylene chloride may also be used for aqueous samples. After appropriate concentration and cleanup, 50 uL of tridecane are added to the extract. Before HRGC-HRMS analysis, 500 pg of a recovery standard ($^{13}C_{12}$-1,2,3,4-TCDD) are added to the extract which is then concentrated to a final volume of 50 uL. A 2-uL aliquot of the concentrated extract is injected into a gas chromatograph with a capillary column interfaced to a high-resolution mass spectrometer capable of rapid multiple ion monitoring at resolutions of at least 10,000 (10 percent valley).

Identification of 2,3,7,8-TCDD is based on the detection of the ions m/z 319.897 and 321.894 at the same GC retention time and within -1 to +3 seconds GC retention time of the internal standard masses of m/z 331.937 and 333.934. Confirmation of 2,3,7,8-TCDD (and of other TCDD isomers) is based on the ion m/z 258.930 which results from loss of COCL by the parent ion.

3. DEFINITIONS

3.1 Concentration calibration solutions -- solutions containing known amounts of the analyte (unlabeled 2,3,7,8-TCDD), the internal standard $^{13}C_{12}$-2,3,7,8-TCDD and the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD; they are used to determine instrument response of the analyte relative to the internal standard and of the internal standard relative to the recovery standard.

3.2 Field blank -- a portion of soil/sediment or water uncontaminated with 2,3,7,8-TCDD and/or other TCDDs.

3.3 Rinsate -- a portion of solvent used to rinse sampling equipment; the rinsate is analyzed to demonstrate that samples have not been contaminated during sampling.

3.4 Internal standard -- $^{13}C_{12}$-2,3,7,8-TCDD, which is added to every sample (except the blanks described in Sections 4.2.1 and 4.2.3 of Exhibit E) and is present at the same concentration in every laboratory method blank, quality control sample, and concentration calibration solution. It is added to the soil/sediment or aqueous sample before extraction and is used to measure the concentration of each analyte. Its concentration is measured in every sample, and percent recovery is determined using an internal standard method.

3.5 Recovery standard -- $^{13}C_{12}$-1,2,3,4-TCDD which is added to every sample (except for the blanks discussed in Sections 4.2.1.A.2 and 4.2.3.6, Exhibit E) extract just before HRGC-HRMS analysis.

3.6 Laboratory method blank -- this blank is prepared in the laboratory through performing all analytical procedures except addition of a sample aliquot to the extraction vessel.

3.7 GC column performance check mixture -- a mixture containing known amounts of selected standards; it is used to demonstrate continued acceptable performance of the capillary column, i.e., separation ($\leq$ 25% valley) of 2,3,7,8-TCDD isomer from all other 21 TCDD isomers and to define the retention time window.

3.8 Performance evaluation sample -- a soil, sediment or aqueous sample containing a known amount of unlabeled 2,3,7,8-TCDD and/or other TCDDs. It is distributed by EPA to potential contractor laboratories who must analyze it and obtain acceptable results before being awarded a contract for sample analyses (see IFB Pre-Award Bid Confirmations). It may also be included as an unspecified ("blind") QC sample in any sample batch submitted to a laboratory for analysis.

3.9 Relative response factor -- response of the mass spectrometer to a known amount of an analyte relative to a known amount of an internal standard.

3.10 Mass resolution check -- standard method used to demonstrate static resolution of 10,000 minimum (10% valley definition).

4. INTERFERENCES

Chemicals which elute from the GC column within ±10 scans of the internal and/or recovery standard (m/z 331.937 and 333.934) and which produce ions at any of the masses used to detect or quantify TCDD are potential interferences. Most frequently encountered potential interferences are other sample components that are extracted along with TCDD, e.g. PCBs, methoxybiphenyls, chlorinated hydroxydiphenylethers, benzylphenylethers, chlorinated naphthalenes, DDE, DDT, etc. The actual incidence of interference by these chemicals depends also upon relative concentrations, mass spectrometric resolution, and chromatographic conditions. Because very low levels of TCDD must be measured, the elimination of interferences is essential. High-purity reagents and solvents must be used and all equipment must be scrupulously cleaned. Laboratory reagent blanks (Exhibit E, Quality Control, Section 4) must be analyzed to demonstrate absence of contamination that would interfere with TCDD measurement. Column chromatographic procedures are used to remove some coextracted sample components; these procedures must be performed carefully to minimize loss of TCDD during attempts to increase its concentration relative to other sample components.

5. SAFETY

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a file of current OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are identified (1-3) (page D-38). 2,3,7,8-TCDD has been identified as a suspected human or mammalian carcinogen. The laboratory is responsible for ensuring that safe handling procedures are followed.

6. APPARATUS AND EQUIPMENT

6.1 High-Resolution Gas Chromatograph/High-Resolution Mass Spectrometer/Data System (HRGC/HRMS/DS)

6.1.1 The GC must be equipped for temperature programming, and all required accessories must be available, such as syringes, gases, and a capillary column. The GC injection port must be designed for capillary columns. The use of splitless injection techniques is recommended. On-column injection techiques can be used but this may severely reduce column lifetime for non-chemically bonded columns. When using the method in this protocol, a 2-uL injection volume is used consistently. With some GC injection ports, however, 1-uL injections may produce improved precision and chromatographic separation. A 1-uL injection volume may be used if adequate sensitivity and precision can be achieved.

NOTE: If 1 uL is used at all as injection volume, the injection volumes for all extracts, blanks, calibration solutions and the performance check sample must be 1 uL.

6.1.2 Gas Chromatograph-Mass Spectrometer Interface

The GC-MS interface may include enrichment devices, such as a glass jet separator or a silicone membrane separator, or the gas chromatograph can be directly coupled to the mass spectrometer source. The interface may include a diverter valve for shunting the column effluent and isolating the mass spectrometer source. All components of the interface should be glass or glass-lined stainless steel. The interface components should be compatible with 300°C temperatures. The GC/MS interface must be appropriately designed so that the separation of 2,3,7,8-TCDD from the other TCDD isomers which is achieved in the gas chromatographic column is not appreciably degraded. Cold spots and/or active surfaces (adsorption sites) in the GC/MS interface can cause peak tailing and peak broadening. It is recommended that the GC column be fitted directly into the MS source. Graphite ferrules should be avoided in the GC injection area since they may adsorb TCDD. Vespel™ or equivalent ferrules are recommended.

6.1.3 Mass Spectrometer

The static resolution of the instrument must be maintained at a minimum 10,000 (10 percent valley). The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with total cycle time (including voltage reset time) of one second or less (Section 8.3.4.1). At a minimum, the following ions which occur at these masses must be monitored: m/z 258.930, 319.897, 321.894, 331.937 and 333.934.

6.1.4 Data System

A dedicated hardware or data system is employed to control the rapid multiple ion monitoring process and to acquire the data. Quantification data (peak areas or peak heights) and SIM traces (displays of intensities of each m/z being monitored as a function of time) must be acquired during the analyses. Quantifications may be reported based upon computer-generated peak areas or upon measured peak heights (chart recording).

NOTE: Detector zero setting must allow peak-to-peak measurement of the noise on the base line.

6.2 GC Columns

For isomer-specific determinations of 2,3,7,8-TCDD, the following two fused silica capillary columns are recommended: a 60-m SP-2330 column and a 50-m CP-Sil 88 column. However, any capillary column which separates 2,3,7,8-TCDD from all other TCDDs may be used for such analyses, but this separation must be demonstrated and documented. Minimum acceptance criteria must be determined per Section 8.1. At the beginning of each 12-hour period (after mass resolution has been demonstrated) during which sample extracts or concentration calibration solutions will be analyzed, column operating conditions must be attained for the required separation on the column to be used for samples. Operating conditions known to produce acceptable results with the recommended columns are shown in Table 2 at the end of this Exhibit.

6.3 Miscellaneous Equipment

6.3.1 Nitrogen evaporation apparatus with variable flow rate.

6.3.2 Balance capable of accurately weighing to 0.01 g.

6.3.3 Centrifuge capable of operating at 2,000 rpm.

6.3.4 Water bath -- equipped with concentric ring cover and capable of being temperature-controlled within ±2°C.

6.3.5 Stainless steel spatulas or spoons.

6.3.6 Stainless steel (or glass) pan large enough to hold contents of 1-pint sample containers.

6.3.7 Glove box.

6.3.8 Drying oven.

6.4 Glassware

6.4.1 Soxhlet apparatus -- all-glass, Kontes 6730-02 or equivalent; 90 mm x 35 mm glass thimble; 500-mL flask; condenser of appropriate size.

6.4.2 Kuderna-Danish apparatus -- 500-mL evaporating flask, 10-mL graduated concentrator tubes with ground-glass stoppers, and 3-ball macro Snyder column (Kontes K-570001-0500, K-503000-0121 and K-569001-0219 or equivalent).

6.4.3 Mini-vials -- 1-mL borosilicate glass with conical-shaped reservoir and screw caps lined with Teflon-faced silicone disks.

6.4.4 Funnels -- glass; appropriate size to accommodate filter paper used to filter jar extract (volume of approximately 170 mL).

6.4.5 Separatory funnel -- 2000 mL with Teflon stopcock.

6.4.6 Continuous liquid-liquid extractors equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication (Hershberg-Wolf Extractor - Ace Glass Company Vineland, NJ, P/N 6841-10 or equivalent).

6.4.7 Chromatographic columns for the silica and alumina chromatography -- 1 cm ID x 10 cm long and 1 cm ID x 30 cm long.

6.4.8 Chromatography column for the Carbopak cleanup -- disposable 5-mL graduated glass pipets, 7 mm ID.

6.4.9 Desiccator.

6.4.10 Glass rods.

NOTE: Reuse of glassware should be minimized to avoid the risk of cross contamination. All glassware that is reused must be scrupulously cleaned as soon as possible after use, applying the following procedure.

Rinse glassware with the last solvent used in it then with high-purity acetone and hexane. Wash with hot water containing detergent. Rinse with copious amounts of tap water and several portions of distilled water. Drain dry and heat in a muffle furnace at 400°C for 15 to 30 minutes. Volumetric glassware should not be heated in a muffle furnace, and some thermally stable materials (such as PCBs) may not be removed by heating in a muffle furnace. In these cases, rinsing with high-purity acetone and hexane may be substituted for muffle furnace heating. After the glassware is dry and cool, rinse with hexane, and store inverted or capped with solvent-rinsed aluminum foil in a clean environment.

7. REAGENTS AND STANDARD SOLUTIONS

7.1 Column Chromatography Reagents

7.1.1 Alumina, acidic -- Extract the alumina in a Soxhlet with methylene chloride for 6 hours (minimum of 3 cycles per hour) and activate it by heating in a foil-covered glass container for 24 hours at 190°C.

7.1.2 Silica gel -- high-purity grade, type 60, 70-230 mesh; extract the silica gel in a Soxhlet with methylene chloride for 6 hours (minimum of 3 cycles per hour) and activate it by heating in a foil-covered glass container for 24 hours at 130°C.

7.1.3 Silica gel impregnated with 40 percent (by weight) sulfuric acid -- add two parts (by weight) concentrated sulfuric acid to three parts (by weight) silica gel (extracted and activated), mix with a glass rod until free of lumps, and store in a screw-capped glass bottle.

7.1.4 Sulfuric acid, concentrated -- ACS grade, specific gravity 1.84.

7.1.5 Graphitized carbon black (Carbopack C or equivalent), surface of approximately 12 m^2/g, 80/100 mesh -- mix thoroughly 3.6 grams Carbopak C and 16.4 grams Celite 545® in a 40-mL vial. Activate at 130° C for six hours. Store in a desiccator.

7.1.6 Celite 545®, reagent grade, or equivalent.

7.2 Membrane filters or filter paper with pore size of ≤25 um; rinse with hexane before use.

7.3 Glass wool, silanized -- extract with methylene chloride and hexane and air-dry before use.

7.4 Desiccating Agents

7.4.1 Sodium sulfate -- granular, anhydrous; before use, extract it with methylene chloride for 6 hours (minimum of 3 cycles per hour) and dry it for ≥4 hours in a shallow tray placed in an oven operated at 120°C. Let it cool in a desiccator.

7.4.2 Potassium carbonate--anhydrous, granular; use as such.

7.5 Solvents -- high purity, distilled in glass: methylene chloride, toluene, benzene, cyclohexane, methanol, acetone, hexane; reagent grade: tridecane.

7.6 Concentration calibration solutions (Table 1) -- five tridecane solutions containing unlabeled 2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD (recovery standard) at varying concentrations, and $^{13}C_{12}$-2,3,7,8-TCDD (internal standard, CASRN 80494-19-5) at a constant concentration must be used to calibrate the instrument. These concentration calibration solutions must be obtained from the Quality Assurance Division, US EPA Environmental Monitoring Systems Laboratory (EMSL-LV), Las Vegas, Nevada. However, additional secondary standards may be obtained from commercial sources, and solutions may be prepared in the contractor laboratory. Traceability of standards must be verified against EPA-supplied standard solutions. Such procedures will be documented by laboratory SOPs as required in IFB Pre-award Bid Confirmations, part 2.f.(4). It is the responsibility of the laboratory to ascertain that the calibration solutions received are indeed at the appropriate concentrations before they are injected into the instrument. Serious overloading of the instrument may occur if the concentration calibration solutions intended for a low-resolution MS are injected into the high-resolution MS.

7.6.1 The five concentration calibration solutions contain unlabeled 2,3,7,8-TCDD and labeled $^{13}C_{12}$-1,2,3,4-TCDD at nominal concentrations of 2.5, 5.0, 10.0, 20.0 and 40.0 pg/uL, respectively, and labeled $^{13}C_{12}$-2,3,7,8-TCDD at a constant nominal concentration of 10.0 pg/uL.

7.6.2 Store the concentration calibration solutions in 1-mL minivials at 4°C.

7.7 Column performance check mixture -- this solventless mixture must be obtained from the Quality Assurance Division, Environmental Monitoring Systems Laboratory, Las Vegas, Nevada, and dissolved by the Contractor in 1 mL tridecane. This solution will then contain the following components (including TCDDs (A) eluting closely to 2,3,7,8-TCDD, and the first- (F) and last-eluting (L) TCDDs when using the columns recommended in Section 6.2) at a concentration of 10 pg/uL of each of these isomers:

Analyte	Approximate Amount Per Ampule
Unlabeled 2,3,7,8-TCDD	10 ng
$^{13}C_{12}$-2,3,7,8-TCDD	10 ng
1,2,3,4-TCDD (A)	10 ng
1,4,7,8-TCDD (A)	10 ng
1,2,3,7-TCDD (A)	10 ng
1,2,3,8-TCDD (A)	10 ng
1,2,7,8-TCDD	10 ng
1,3,6,8-TCDD (F)	10 ng
1,2,8,9-TCDD (L)	10 ng

7.8 Sample fortification solution -- an isooctane solution containing the internal standard at a nominal concentration of 5 pg/uL.

7.9 Recovery standard spiking solution -- an isooctane solution containing the recovery standard at a nominal concentration of 100 pg/uL. Five uL of this solution will be spiked into the extract before HRGC/HRMS analysis.

7.10 Internal standard spiking solution -- an isooctane solution containing the internal standard at a nominal concentration of 100 pg/uL. Five uL of this solution will be added to a fortified field blank extract (Section 4.2.1.A.2, Exhibit E).

8. SYSTEM PERFORMANCE CRITERIA

System performance criteria are presented in two sections. One section deals with GC column performance criteria while the other section consists of initial calibration criteria. The laboratory may use either of the recommended columns described in Section 6.2. It must be documented that all applicable system performance criteria specified in Sections 8.1, 8.2 and 8.3 have been met before analysis of any sample is performed. Table 2 provides recommended conditions that can be used to satisfy the required criteria. Table 3 provides a typical 12-hour analysis sequence.

8.1 GC Column Performance

8.1.1 Inject 2 uL (Section 6.1.1) of the column performance check solution (Section 7.7) and acquire selected ion monitoring (SIM) data for m/z 258.930, 319.897, 321.894, 331.937 and 333.934 within a total cycle time of ≤ 1 second (Section 8.3.4.1).

8.1.2 The chromatographic peak separation between 2,3,7,8-TCDD and the peaks representing any other TCDD isomers must be resolved with a valley of ≤ 25 percent, where

$$\text{Valley Percent} = (x/y)(100)$$

x = measured as in Figures 1 and 2

y = the peak height of 2,3,7,8-TCDD.

It is the responsibility of the laboratory to verify the conditions suitable for the appropriate resolution of 2,3,7,8-TCDD from all other TCDD isomers. The column performance check solution also contains the TCDD isomers eluting first and last under the analytical conditions specified in this protocol thus defining the retention time window for total TCDD determination. The peaks representing 2,3,7,8-TCDD, the first and the last eluting TCDD isomers must be labeled and identified as such on the chromatograms.

8.2 Mass Spectrometer Performance

8.2.1 The mass spectrometer must be operated in the electron (impact) ionization mode. Static mass resolution of at least 10,000 (10 percent valley) must be demonstrated before any analysis of a set of samples is performed (Section 8.2.2). Static resolution checks must be performed at the beginning and at the end of each 12-hour period of operation. However, it is recommended that a visual check (i.e., not documented) of the static resolution be made using the peak matching unit before and after each analysis.

8.2.2 Chromatography time for TCDD may exceed the long-term mass stability of the mass spectrometer and thus mass drift correction is mandatory. A reference compound (high boiling PFK is recommended) is introduced into the mass spectrometer. An acceptable lock mass ion at any mass between m/z 250 and m/z 334 (m/z 318.979 from PFK is recommended) must be used to monitor and correct mass drifts.

NOTE: Excessive PFK may cause background noise problems and contamination of the source resulting in an increase in "downtime" for source cleaning.

Using a PFK molecular leak, tune the instrument to meet the minimum required mass resolution of 10,000 (10% valley) at m/z 254.986 (or any other mass reasonably close to m/z 259). Calibrate the voltage sweep at least across the mass range m/z 259 to m/z 334 and verify that m/z 330.979 from PFK (or any other mass close to m/z 334) is measured within ±5 ppm (i.e., 1.7 mmu) using m/z 254.986 as a reference. Documentation of the mass resolution must then be accomplished by recording the peak profile of the PFK reference peak m/z 318.979 (or any other reference peak at a mass close to m/z 320/322). The format of the peak profile representation must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale (amu or ppm per division). The result of the peak width measurement (performed at 5 percent of the maximum) must appear on the hard copy and cannot exceed 31.9 mmu or 100 ppm.

8.3 Initial Calibration

Initial calibration is required before any samples are analyzed for 2,3,7,8-TCDD. Initial calibration is also required if any routine calibration does not meet the required criteria listed in Section 8.6.

8.3.1 All concentration calibration solutions listed in Table 1 must be utilized for the initial calibration.

8.3.2 Tune the instrument with PFK as described in Section 8.2.2.

8.3.3 Inject 2 uL of the column performance check solution (Section 7.7) and acquire SIM mass spectral data for m/z 258.930, 319.897, 321.894, 331.937 and 333.934 using a total cycle time of ≤ 1 second (Section 8.3.4.1). The laboratory must not perform any further analysis until it has been demonstrated and documented that the criterion listed in Section 8.1.2 has been met.

8.3.4 Using the same GC (Section 8.1) and MS (Section 8.2) conditions that produced acceptable results with the column performance check solution, analyze a 2-uL aliquot of each of the 5 concentration calibration solutions in triplicate with the following MS operating parameters.

8.3.4.1 Total cycle time for data acquisition must be ≤ 1 second. Total cycle time includes the sum of all the dwell times and voltage reset times.

8.3.4.2 Acquire SIM data for the following selected characteristic ions:

m/z	Compound
258.930	TCDD - COCl
319.897	Unlabeled TCDD
321.894	Unlabeled TCDD
331.937	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD
333.934	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD

8.3.4.3 The ratio of integrated ion current for m/z 319.897 to m/z 321.894 for 2,3,7,8-TCDD must be between 0.67 and 0.90.

8.3.4.4 The ratio of integrated ion current for m/z 331.937 to m/z 333.934 for $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD must be between 0.67 and 0.90.

8.3.4.5 Calculate the relative response factors for unlabeled 2,3,7,8-TCDD [RRF(I)] relative to $^{13}C_{12}$-2,3,7,8-TCDD and for labeled $^{13}C_{12}$-2,3,7,8-TCDD [RRF(II)] relative to $^{13}C_{12}$-1,2,3,4-TCDD as follows:

$$\mathrm{RRF(I)} = \frac{A_x \cdot Q_{IS}}{Q_x \cdot A_{IS}}$$

$$\mathrm{RRF(II)} = \frac{A_{IS} \cdot Q_{RS}}{Q_{IS} \cdot A_{RS}}$$

where

A_x = sum of the integrated ion abundances of m/z 319.897 and m/z 321.894 for unlabeled 2,3,7,8-TCDD.

A_{IS} = sum of the integrated ion abundances of m/z 331.937 and m/z 333.934 for $^{13}C_{12}$-2,3,7,8-TCDD.

A_{RS} = sum of the integrated ion abundances for m/z 331.937 and m/z 333.934 for $^{13}C_{12}$-1,2,3,4-TCDD.

Q_{IS} = Quantity of $^{13}C_{12}$-2,3,7,8-TCDD injected (pg).

Q_{RS} = quantity of $^{13}C_{12}$-1,2,3,4-TCDD injected (pg).

Q_x = quantity of unlabeled 2,3,7,8-TCDD injected (pg).

RRF is a dimensionless quantity; the units used to express Q_{IS}, Q_{RS} and Q_x must be the same.

8.4 Criteria for Acceptable Calibration

The criteria listed below for acceptable calibration must be met before analysis of any sample is performed.

8.4.1 The percent relative standard deviation (RSD) for the response factors from each of the triplicate analyses for both unlabeled and $^{13}C_{12}$-2,3,7,8-TCDD must be less than ± 20 percent.

8.4.2 The variation of the 5 mean RRFs for unlabeled 2,3,7,8-TCDD obtained from the triplicate analyses must be less than ± 20 percent RSD.

8.4.3 SIM traces for 2,3,7,8-TCDD must present a signal-to-noise ratio of ≥ 2.5 for m/z 258.930 and ≥ 10 for m/z 321.894.

8.4.4 SIM traces for $^{13}C_{12}$-2,3,7,8-TCDD must present a signal-to-noise ratio ≥ 10 for 333.934.

8.4.5 Isotopic ratios (Sections 8.3.4.3 and 8.3.4.4) must be within the allowed range.

NOTE: If the criteria for acceptable calibration listed in Sections 8.4.1 and 8.4.2 have been met, the RRF can be considered independent of the analyte quantity for the calibration concentration range. The mean RRF from 5 triplicate determinations for unlabeled 2,3,7,8-TCDD and for $^{13}C_{12}$-2,3,7,8-TCDD will be used for all calculations until routine calibration criteria (Section 8.6) are no longer met. At such time, new mean RRFs will be calculated from a new set of five triplicate determinations.

8.5 Routine Calibrations

Routine calibrations must be performed at the beginning of a 12-hour period after successful mass resolution and GC column performance check runs.

8.5.1 Inject 2 uL of the concentration calibration solution which contains 5.0 pg/uL of unlabeled 2,3,7,8-TCDD, 10.0 pg/uL of $^{13}C_{12}$-2,3,7,8-TCDD and 5.0 pg/uL $^{13}C_{12}$-1,2,3,4-TCDD. Using the same GC/MS/DS conditions as used in Sections 8.1, 8.2 and 8.3, determine and document acceptable calibration as provided in Section 8.6.

8.6 Criteria for Acceptable Routine Calibration

The following criteria must be met before further analysis is performed. If these criteria are not met, corrective action must be taken and the instrument must be recalibrated.

8.6.1 The measured RRF for unlabeled 2,3,7,8-TCDD must be within ±20 percent of the mean values established (Section 8.3.4.6) by triplicate analyses of concentration calibration solutions.

8.6.2 The measured RRF for $^{13}C_{12}$-2,3,7,8-TCDD must be within ±20 percent of the mean value established by triplicate analysis of the concentration calibration solutions (Section 8.3.4.6).

8.6.3 Isotopic ratios (Sections 8.3.4.3 and 8.3.4.4) must be within the allowed range.

8.6.4 If one of the above criteria is not satisfied, a second attempt can be made before repeating the entire initialization process (Section 8.3).

NOTE: An initial calibration must be carried out whenever any HRCC solution is replaced.

9. QUALITY CONTROL PROCEDURES

See Exhibit E for QA/QC requirements.

10. SAMPLE PRESERVATION AND HANDLING

10.1 Chain-of-custody procedures -- see Exhibit G.

10.2 Sample Preservation

10.2.1 When received, each soil or sediment sample will be contained in a 1-pint glass jar surrounded by vermiculite in a sealed metal paint can. Until a portion is to be removed for analysis, store the sealed paint cans in a locked limited-access area where the temperature is maintained between 25° and 35°C. After a portion of a sample has been removed for analysis, return the remainder of the sample to its original container and store as stated above.

10.2.2 Each aqueous sample will be contained in a 1-liter glass bottle. The bottles with the samples are stored at 4°C in a refrigerator located in a locked limited-access area.

10.2.3 To avoid photodecomposition, protect samples from light.

10.3 Sample Handling

CAUTION: Finely divided soils contaminated with 2,3,7,8-TCDD are hazardous because of the potential for inhalation or ingestion of particles containing 2,3,7,8-TCDD. Such samples should be handled in a confined environment (i.e., a closed hood or a glove box).

10.3.1 Pre-extraction sample treatment

10.3.1.1 Homogenization -- Although sampling personnel will attempt to collect homogeneous samples, the contractor shall examine each sample and judge if it needs further mixing.

NOTE: Contractor personnel have the responsibility to take a representative sample portion; this responsibility entails efforts to make the sample as homogeneous as possible. Stirring is recommended when possible.

10.3.1.2 Centrifugation -- When a soil or sediment sample contains an obvious liquid phase, it must be centrifuged to separate the liquid from the solid phase. Place the entire sample in a suitable centrifuge bottle and centrifuge for 10 minutes at 2000 rpm. Remove the bottle from the centrifuge. With a disposable pipet, remove the liquid phase and discard it. Mix the solid phase with a stainless steel spatula and remove a portion to be air-dried, weighed and analyzed. Return the remaining solid portion to the original sample bottle and store it as described in 10.2.1.

CAUTION: The removed liquid may contain TCDD and should be disposed as a liquid waste.

10.3.1.3 Weigh between 9.5 and 10.5 g of the air-dried soil sample ($\pm$0.5 g) to 3 significant figures. Dry it to constant weight at 100°C. Allow the sample to cool in a desiccator. Weigh the dried soil to 3 significant figures. Calculate and report percent moisture on Form B-1.

11. SAMPLE EXTRACTION

11.1 Soil Extraction

11.1.1 Immediately before use, the Soxhlet apparatus is charged with 200 to 250 mL benzene which is then refluxed for 2 hours. The apparatus is allowed to cool, disassembled and the benzene removed and retained as a blank for later analysis if required.

11.1.2 Accurately weigh to 3 significant figures a 10-g (9.50 g to 10.50 g) portion of the wet soil or sediment sample. Mix 100 uL of the sample fortification solution (Section 7.8) with 1.5 mL of acetone (500 pg of $^{13}C_{12}$-2,3,7,8-TCDD) and deposit the entire mixture in small portions on several sites on the surface of the soil or sediment.

11.1.3 Add 10 g anhydrous sodium sulfate and mix thoroughly using a stainless steel spoon spatula.

11.1.4 After breaking up any lumps, place the soil-sodium sulfate mixture in the Soxhlet apparatus using a glass wool plug (the use of an extraction thimble is optional). Add 200 to 250 mL benzene to the Soxhlet apparatus and reflux for 24 hours. The solvent must cycle completely through the system at least 3 times per hour.

11.1.5 Transfer the extract to a Kuderna-Danish apparatus and concentrate to 2 to 3 uL. Rinse the column and flask with 5 mL benzene and collect the rinsate in the concentrator tube. Reduce the volume in the concentrator tube to 2 to 3 uL. Repeat this rinsing and concentrating operation twice more. Remove the concentrator tube from the K-D apparatus and carefully reduce the extract volume to approximately 1 mL with a stream of nitrogen using a flow rate and distance such that gentle solution surface rippling is observed.

NOTE: Glassware used for more than one sample must be carefully cleaned between uses to prevent cross-contamination (Note on page D-10).

11.2 Extraction of Aqueous Samples

11.2.1 Mark the water meniscus on the side of the 1-L sample bottle for later determination of the exact sample volume. Pour the entire sample (approximately 1 L) into a 2-L separatory funnel.

11.2.2 Mix 100 uL of the sample fortification solution with 1.5 mL of acetone (500 pg of $^{13}C_{12}$-2,3,7,8-TCDD) and add the mixture to the sample in the separatory funnel.

NOTE: A continuous liquid-liquid extractor may be used in place of a separatory funnel.

11.2.3 Add 60 mL methylene chloride to the sample bottle, seal and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If the emulsion interface between layers is more than one-third the volume of the solvent layer, the analyst must employ mechanical techniques to complete the phase separation. Collect the methylene chloride (3 x 60 mL) directly into a 500 mL Kuderna-Danish concentrator (mounted with a 10 mL concentrator tube) by passing the sample extracts through a filter funnel packed with a glass wool plug and 5 g of anhydrous sodium sulfate. After the third extraction, rinse the sodium sulfate with an additional 30 mL of methylene chloride to ensure quantitiative transfer.

11.2.4 Attach a Snyder column and concentrate the extract until the apparent volume of the liquid reaches 1 mL. Remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column, add 50 mL benzene, reattach the Snyder column and concentrate to approximately 1 mL. Rinse the flask and the lower joint with 1 to 2 mL benzene. Concentrate the extract to 1.0 mL under a gentle stream of nitrogen.

11.2.5 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.3 Cleanup Procedures - Column Cleanup

11.3.1 Prepare an acidic silica column as follows: Pack a 1 cm x 10 cm chromatographic column with a glass wool plug, a layer (1 cm) of Na_2SO_4/K_2CO_3(1:1), 1.0 g silica gel (Section 7.1.2) and 4.0 g of 40-percent w/w sulfuric acid-impregnated silica gel (Section 7.1.3). Pack a second chromatographic column (1 cm x 30 cm) with a glass wool plug, 6.0 g acidic alumina (Section 7.1.1) and top with a 1-cm layer of sodium sulfate (Section 7.4). Add hexane to the columns until they are free of channels and air bubbles.

11.3.2 Quantitatively transfer the benzene extract (1 mL) from the concentrator tube to the top of the silica gel column. Rinse the concentrator tube with two 0.5-mL portions of hexane. Transfer the rinses to the top of the silica gel column.

11.3.3 Elute the extract from the silica gel column with 90 mL hexane directly into a Kuderna-Danish concentrator. Concentrate the eluate to 0.5 mL, using nitrogen blow-down as necessary.

11.3.4 Transfer the concentrate (0.5 mL) to the top of the alumina column. Rinse the K-D assembly with two 0.5-mL portions of hexane and transfer the rinses to the top of the alumina columns. Elute the alumina column with 18 mL hexane until the hexane level is just below the top of the sodium sulfate. Discard the eluate. Columns must not be allowed to reach dryness (i.e., a solvent "head" must be maintained.)

11.3.5 Place 30 mL of 20-percent (v/v) methylene chloride in hexane on top of the alumina and elute the TCDDs from the column. Collect this fraction in a 50-mL Erlenmeyer flask.

11.3.6 Certain extracts, even after cleanup by column chromatography, contain interferences which preclude determination of TCDD at low parts-per-trillion levels. Therefore, a cleanup step is included using activated carbon which selectively retains planar molecules such as TCDD. The TCDDs are then removed from the carbon by elution with toluene. Proceed as follows: Prepare a 18-percent Carbopak C/Celite 545® mixture by thoroughly mixing 3.6 grams Carbopak C (80/100 mesh) and 16.4 grams Celite 545® in a 40-mL vial. Activate at 130°C for 6 hours. Store in a desiccator. Cut off a clean 5-mL disposable glass pipet at the 4-mL mark. Insert a plug of glass wool (Section 7.3) and push to the 2-mL mark. Add 340 mg of the activated Carbopak/Celite mixture followed by another glass wool plug. Using two glass rods, push both glass wool plugs simultaneously towards the Carbopak/Celite mixture and gently compress the Carbopak/Celite plug to a length of 2 to 2.5 cm. Preelute the column with 2 mL toluene followed by 1 mL of 75:20:5 methylene chloride/methanol/benzene, 1 mL of 1:1 cyclohexane in methylene chloride, and 2 mL hexane. The flow rate should be less than 0.5 mL $min.^{-1}$. While the column is still wet with hexane, add the entire eluate (30 mL) from the alumina column (Section 11.3.5) to the top of the column. Rinse the Erlenmeyer flask which contained the extract twice with 1 mL hexane and add the rinsates to the top of the column. Elute the column sequentially with two 1-mL aliquots hexane, 1 mL of 1:1 cyclohexane in methylene chloride, and 1 mL of 75:20:5 methylene chloride/ methanol/benzene. Turn the column upside down and elute the TCDD fraction with 6 mL toluene into a concentrator tube. Warm the tube to approximately 60°C and reduce the toluene volume to approximately 1 mL using a stream of nitrogen. Carefully transfer the residue into a 1-mL mini-vial and again at elevated temperature, reduce the volume to about 100 uL using a stream of nitrogen. Rinse the concentrator tube with 3 washings using 200 uL of 1% toluene in CH_2Cl_2. Add 50 uL tridecane and store the sample in a refrigerator until GC/MS analysis is performed.

12. ANALYTICAL PROCEDURES

12.1 Remove the sample extract or blank from storage, allow it to warm to ambient laboratory temperature and add 5 uL recovery standard solution. With a stream of dry, purified nitrogen, reduce the extract/blank volume to 50 uL.

12.2 Inject a 2-uL aliquot of the extract into the GC, operated under the conditions previously used (Section 8.1) to produce acceptable results with the performance check solution.

12.3 Acquire SIM data according to 12.3.1. Use the same acquisition time and MS operating conditions previously used (Section 8.3.4) to determine the relative response factors.

12.3.1 Acquire SIM data for the following selected characteristic ions:

m/z	Compound
258.930	TCDD - COCl
319.897	Unlabeled TCDD
321.894	Unlabeled TCDD
331.937	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD
333.934	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD

12.4 Identification Criteria

12.4.1 The retention time (RT) (at maximum peak height) of the sample component m/z 319.897 must be within -1 to +3 seconds of the retention time of the peak for the isotopically labeled internal standard at m/z 331.937 to attain a positive identification of 2,3,7,8-TCDD. Retention times of other tentatively identified TCDDs must fall within the RT window established by analyzing the column performance check solution (Section 8.1). Retention times are required for all chromatograms.

12.4.2 The ion current responses for m/z 258.930, 319.897 and 321.894 must reach maximum simultaneously (± 1 scan), and all ion current intensities must be ≥ 2.5 times noise level for positive identification of a TCDD.

12.4.3 The integrated ion current at m/z 319.897 must be between 67 and 90 percent of the ion current response at m/z 321.894.

12.4.4 The integrated ion current at m/z 331.937 must be between 67 and 90 percent of the ion current response at m/z 333.934.

12.4.5 The integrated ion currents for m/z 331.937 and 333.934 must reach their maxima within ± 1 scan.

12.4.6 The recovery of the internal standard $^{13}C_{12}$-2,3,7,8-TCDD must be between 40 and 120 percent.

13. CALCULATIONS

13.1 Calculate the concentration of 2,3,7,8-TCDD (or any other TCDD isomer) using the formula:

$$C_X = \frac{A_X \cdot Q_{IS}}{A_{IS} \cdot W \cdot \overline{RRF}(I)}$$

where:

C_X = unlabeled 2,3,7,8-TCDD (or any other unlabeled TCDD isomer) concentration in pg/g for soil/sediment and pg/L for aqueous samples.

A_X = sum of the integrated ion abundances determined for m/z 319.897 and 321.894.

A_{IS} = sum of the integrated ion abundances determined for m/z 331.937 and 333.934 of $^{13}C_{12}$-2,3,7,8-TCDD (IS = internal standard).

Q_{IS} = quantity (in picograms) of $^{13}C_{12}$-2,3,7,8-TCDD added to the sample before extraction (Q_{IS} = 500 pg).

W = weight (in grams) of dry soil or sediment sample or volume of aqueous sample (in liters).

$\overline{RRF}(I)$ = calculated mean relative response factor for unlabeled 2,3,7,8TCDD relative to $^{13}C_{12}$-2,3,7,8-TCDD. This represents the grand mean of the RRF(I)'s obtained in Section 8.3.4.5.

13.2 Calculate the recovery of the internal standard $^{13}C_{12}$-2, 3,7,8-TCDD, measured in the sample extract, using the formula:

$$\text{Internal standard percent recovery} = \frac{A_{IS} \cdot Q_{RS}}{A_{RS} \cdot \overline{RRF}(II) \cdot Q_{IS}} \times 100$$

where A_{IS} and Q_{IS} have the same definitions as above (Section 13.1)

A_{RS} = sum of the integrated ion abundances determined for m/z 331.937 and 333.934 of $^{13}C_{12}$-1,2,3,4-TCDD (RS = recovery standard).

Q_{RS} = quantity (in picograms) of $^{13}C_{12}$-1,2,3,4-TCDD added to the sample residue before HRGC-HRMS analysis.

(Q_{RS} = 500 pg).

$\overline{RRF}(II)$ = calculated mean relative response factor for labeled $^{13}C_{12}$-2,3,7,8-TCDD relative to $^{13}C_{12}$-1,2,3,4-TCDD. This represents the grand mean of the RRF(II)'s calculated in Section 8.3.4.5.

13.3 If the calculated concentration of unlabeled 2,3,7,8-TCDD exceeds 200 pg/g for soils or sediments, or 2000 pg/L for aqueous samples, the linear range of response vs. concentration may have been exceeded and a smaller portion of that sample must be analyzed. Accurately weigh to three significant figures a 1-g portion of the wet soil/sediment. Add the sample fortification solution (Section 11.1.2), extract and analyze as discussed for the 10-g sample. Similarly, add the sample fortification solution (Section 11.2.2) to 100 mL of the aqueous sample, extract and analyze.

13.4 Total TCDD concentration -- all positively identified isomers of TCDD must be within the RT window and meet all identification criteria listed in Sections 12.4.2, 12.4.3 and 12.4.4. Use the expression in Section 13.1 to calculate the concentrations of the other TCDD isomers, with C_x becoming the concentration of any unlabeled TCDD isomer.

$C_{\text{Total TCDD}}$ = Sum of the concentrations of the individual TCDDs.

13.5 Estimated Detection Limit -- For samples in which no unlabeled 2,3,7,8-TCDD was detected, calculate the estimated minimum detectable concentration. The background area is determined by integrating the ion abundances for m/z 319.897 and 321.894 in the appropriate region of the selected ion monitoring trace, multiplying that area by 2.5, and relating the product area to an estimated concentration that would produce that product area.

Use the formula:

$$C_E = \frac{(2.5) \cdot (A_x) \cdot (Q_{IS})}{(A_{IS}) \cdot (\overline{RRF}(I)) \cdot (W)}$$

where

C_E = estimated concentration of unlabeled 2,3,7,8-TCDD required to produce Ax.

A_x = sum of integrated ion abundance for m/z 319.897 and 321.894 in the same group of ≥ 5 scans used to measure A_{IS}.

A_{IS} = sum of integrated ion abundance for the appropriate ion characteristic of the internal standard, m/z 331.937 and m/z 333.934.

Q_{IS}, $\overline{RRF}(I)$, and W retain the definitions previously stated in Section 13.1. Alternatively, if peak height measurements are used for quantification, measure the estimated detection limit by the peak height of the noise in the TCDD RT window.

13.6 The relative percent difference (RPD) is calculated as follows:

$$RPD = \frac{|S_1 - S_2|}{\text{Mean Concentration}} = \frac{|S_1 - S_2|}{(S_1 + S_2)/2} \times 100$$

S_1 and S_2 represent sample and duplicate sample results.

References

1. "Carcinogens - Working with Carcinogens", Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

2. "OSHA Safety and Health Standards, General Industry" (29 CFR1910), Occupational Safety and Health Administration, OSHA 2206 (Revised January 1976).

3. "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition 1979.

TABLE 1. COMPOSITION OF CONCENTRATION CALIBRATION SOLUTIONS

	Recovery Standard $^{13}C_{12}$-1,2,3,4-TCDD	Analyte 2,3,7,8-TCDD	Internal Standard $^{13}C_{12}$-2,3,7,8-TCDD
HRCC1	2.5 pg/uL	2.5 pg/uL	10.0 pg/uL
HRCC2	5.0 pg/uL	5.0 pg/uL	10.0 pg/uL
HRCC3	10.0 pg/uL	10.0 pg/uL	10.0 pg/uL
HRCC4	20.0 pg/uL	20.0 pg/uL	10.0 pg/uL
HRCC5	40.0 pg/uL	40.0 pg/uL	10.0 pg/uL

Sample Fortification Solution

5.0 pg/uL of $^{13}C_{12}$-2,3,7,8-TCDD

Recovery Standard Spiking Solution

100 pg/uL $^{13}C_{12}$-1,2,3,4-TCDD

Field Blank Fortification Solutions

A) 5.0 pg/uL of unlabeled 2,3,7,8-TCDD

B) 5.0 pg/uL of unlabeled 1,2,3,4-TCDD

Internal Standard Spiking Solution

100 pg/uL of $^{13}C_{12}$-2,3,7,8-TCDD
(Used only in Section 4.2.1.A.2, Exhibit E)

TABLE 2. RECOMMENDED GC OPERATING CONDITIONS

Column coating	SP-2330	CP-SIL 88
Film thickness	0.2 um	0.22 um
Column dimensions	60 m x 0.24 mm	50 m x 0.22 mm
Helium linear velocity	28-29 cm/sec at 240°C	28-29 cm/sec at 240°C
Initial temperature	70°C	45°C
Initial time	4 min	3 min
Temperature program	Rapid increase to 200°C 200°C to 250°C at 4°C/min	Rapid increase to 190°C 190°C to 240°C at 5°C/min
2,3,7,8-TCDD retention time	24 min	26 min

TABLE 3. TYPICAL 12-HOUR SEQUENCE FOR 2,3,7,8-TCDD ANALYSIS

1.	Static mass resolution check	10/20/84	0700 hrs.
2.	Column performance check	10/20/84	0730 hrs.
3.	HRCC2	10/20/84	0800 hrs.
4.	Sample 1 through Sample "N"	10/20/84	0830 hrs.
5.	Column performance check	10/20/84	1800 hrs.
6.	Static mass resolution check	10/20/84	1830 hrs.

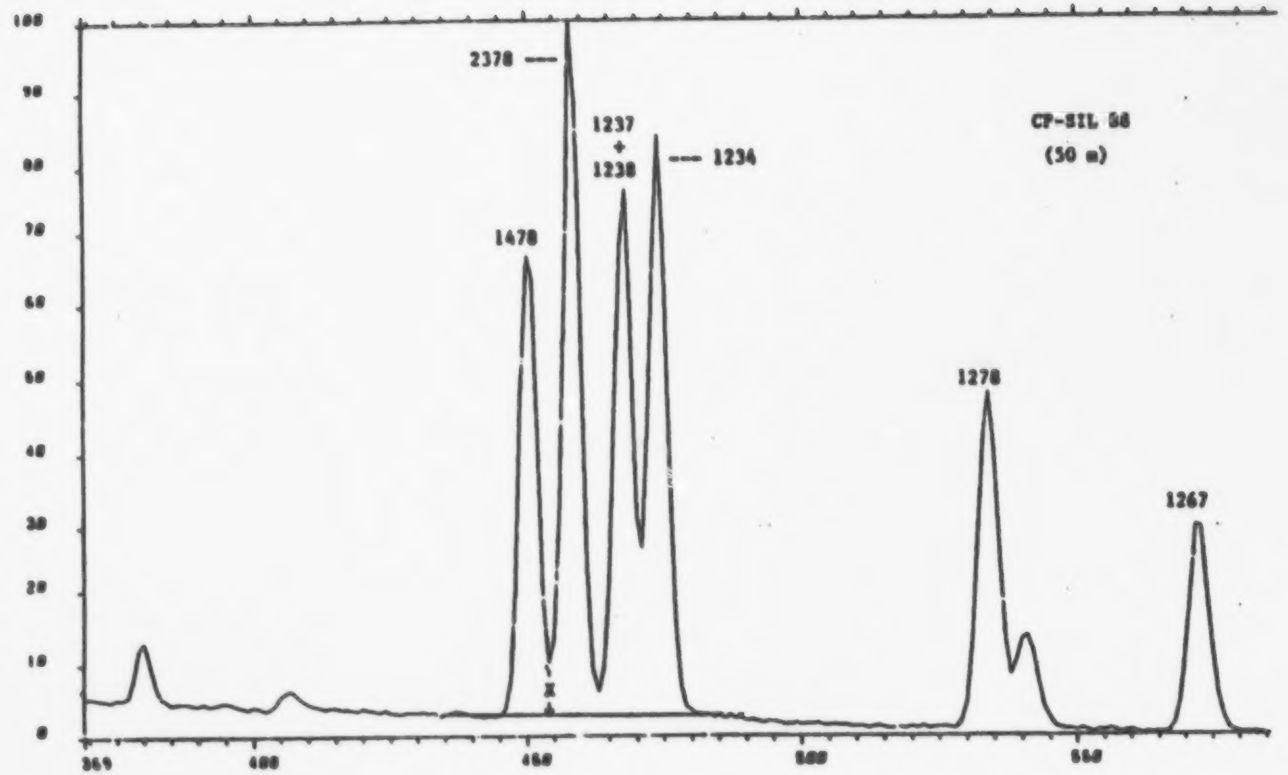

Figure 1. Selected ion current profile for m/z 320 and 322 produced by MS analysis for performance check solution using a 50-m CP Sil-88 fused silica capillary column and conditions listed in Table 2.

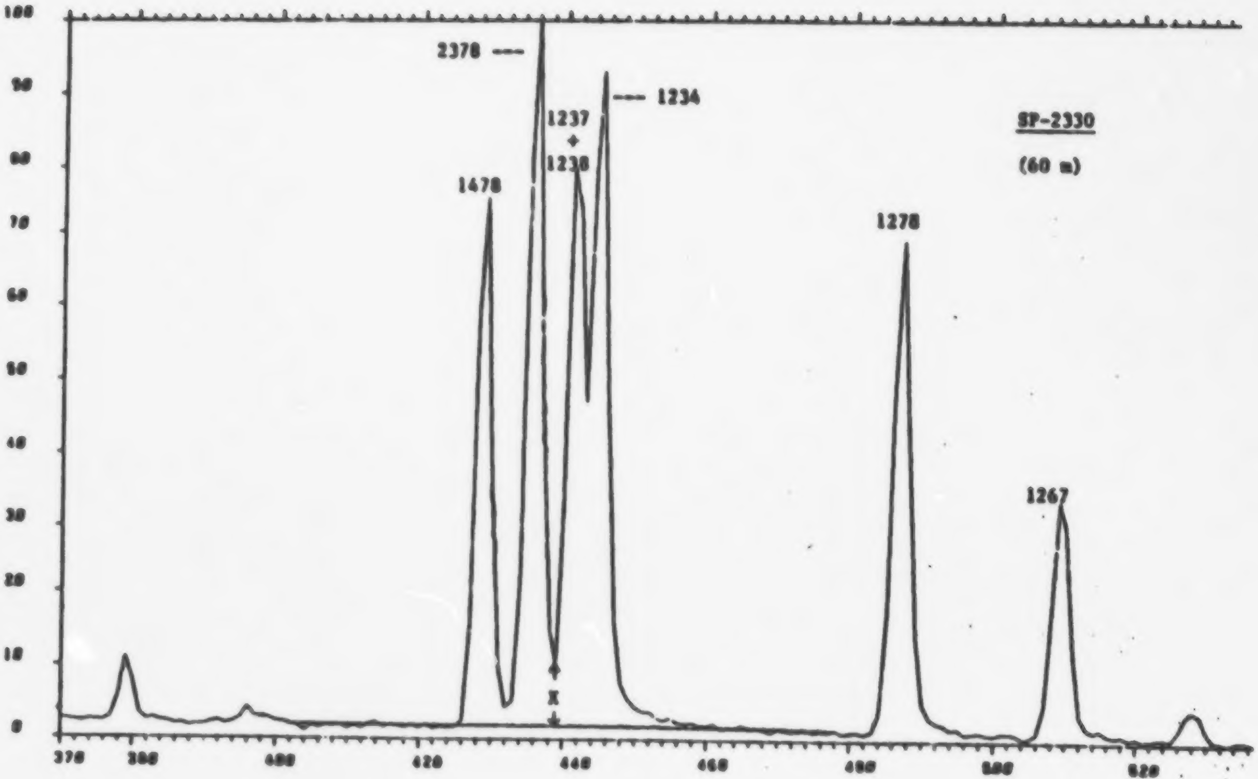

Figure 2. Selected ion current profile for m/z 320 and 322 produced by MS analysis of performance check solution using a 60-m SP-2330 fused silica capillary column and conditions listed in Table 2.

APPENDIX B

PROPOSED ANALYTICAL PROTOCOL

for the Determination of

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and Total
TCDDs in Soil/Sediment and Water by High-Resolution
Gas Chromatography/High-Resolution Mass Spectrometry

December 1, 1985

This analytical protocol has been written in the format used in the Superfund program, as "Exhibit D" of a Statement of Work which in turn is part of an Invitation-for-Bid package under the Superfund Contract Laboratory Program. Also included are other exhibits listed below for the Statement of Work which have been tailored to meet the specific requirements of this protocol:

EXHIBIT B: Reporting Requirements and Deliverables

EXHIBIT C: Sample Rerun Requirements

EXHIBIT D: Analytical Method

EXHIBIT E: Quality Assurance/Quality Control Requirements

This protocol (Protocol B) is a modification of the protocol presented as Appendix A (Protocol A). Examination of the results from the single-laboratory evaluation of Protocol A had shown that the minimum amount of 2,3,7,8-TCDD that could be quantified under the conditions specified in Protocol A was 5 pg. However, a requirement existed to lower the quantitation limits to 2 ppt for soil and sediment samples and to 20 ppq for aqueous samples. The sample size should stay at 10 g for soil and sediments and at 1 L for aqueous samples, since the effect of larger sample sizes on the extract cleanup efficiencies is not known. Also, the range of the method should overlap with the 1-ppb lower limit of the low-resolution analytical method for TCDD used in the Superfund Contract Laboratory Program without necessitating second extractions for samples containing higher levels of TCDDs.

After careful evaluation by EMSL-LV of the requirements and the options, the following protocol changes were made:

- In Protocol B, the following calibration solutions will be used:

HRCC1:	2 pg/µL	2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD
	10 pg/µL	$^{13}C_{12}$-2,3,7,8-TCDD
HRCC2:	10 pg/µL	2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD
	10 pg/µL	$^{13}C_{12}$-2,3,7,8-TCDD
HRCC3:	50 pg/µL	2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD
	10 pg/µL	$^{13}C_{12}$-2,3,7,8-TCDD

HRCC4: 100 pg/µL 2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD
10 pg/µL $^{13}C_{12}$-2,3,7,8-TCDD

- In Protocol B, the final extract volume will be 10 µL. The decision to select a final volume of 10 µL was necessary in order to comply with the above requirements. It is realized that such a small volume may pose technical difficulties for the analyst.

- In Protocol B, the fortification level of the internal standard $^{13}C_{12}$-2,3,7,8-TCDD was raised from 500 pg/sample to 1,000 pg/sample. This allows analysis of soil and sediment samples containing between 100 ppt and 1.2 ppb of any TCDD isomer and of water samples containing between 1 ppt and 12 ppt of any TCDD isomer by diluting a 2-µL aliquot of the remaining extract concentrate by a factor of 12 with a solution of the recovery standard (100 pg/µL of $^{13}C_{12}$-1,2,3,4-TCDD in tridecane). Recoveries will be reported using the data generated from the first injection. Thus, the decision to dilute an aliquot of the 10-µL final extract will not be based on the concentration of 2,3,7,8-TCDD or total TCDD in the sample, but on the concentration of the most abundant TCDD isomer in the 10-µL final extract volume. This will eliminate unnecessary dilutions of the sample extract and analyses for samples containing between 100 ppt and 250 ppt for soil and sediment and 1 ppt and 2.5 ppt for water samples of a TCDD isomer, but for which the recoveries were low.

EXHIBIT B

Reporting Requirements and Deliverables

1. SCOPE AND APPLICATION

The Contractor shall provide reports and other deliverables as specified in the Contract Reporting Schedule. These reports are described below. All reports shall be submitted in legible form or resubmission shall be required. All reports and documentation required, including selected ion current profiles (also called selected ion monitoring traces), shall be clearly labeled with the Sample Management Office Case number and associated Sample/Traffic Report number(s). If documentation is submitted without the required identification, as specified above, resubmission shall be required.

The Contract Reporting Schedule (Section 2) specifies the numbers of copies required, the delivery schedule and the distribution of all required deliverables.

1.1 Sample data package -- Hard copy analytical data and documentation are required as described below.

NOTE: This analytical protocol is designed for the receipt and analysis of samples by batches. Therefore, it is desired that sample data from samples in the same batch be reported together, i.e., on the same reporting form. However, contract accounting and billing are based on the sample unit.

1.1.1 Case narrative: Contains the Case number, Dioxin Shipment Record numbers, Contract number and detailed documentation of any quality control, sample, shipment and/or analytical problems encountered in a specific Case. Also included should be documentation of any internal decision tree process used along with a summary of corrective actions taken. The Case narrative must be signed in original signature by the Laboratory Manager or his designate.

1.1.2 Results of initial triplicate analyses of four (4) concentration calibration solutions (Form H-2), routine calibration solutions, (Form H-3), including all selected ion current profiles or selected ion monitoring (SIM) traces, calculated relative response factors (RRF), and computer-generated quantification reports (or manual calculations).

1.1.3 Completed data reporting sheets (Forms H-1, H-4, and H-5, H-8 and H-9) with appropriate SIM traces (including the lock mass SIM traces). Data results for levels less than 10 ppt but above the quantitation limit (Section 1.1, Exhibit D) attained for that sample shall be reported to two (2) significant figures; results greater than 10 ppt shall be reported to three (3) significant figures. Apply the rounding rules found in Section 7.2.2, "Handbook for Analytical Quality Control in Water and Wastewater Laboratories," EPA-600/4-79-019. Each SIM trace shall include computer-generated header information indicating instrumental (GC and MS) operating parameters during data acquisition. When samples are analyzed more than once, all sample data shall be reported. Rejected sample runs must be separated and attached to the back of the data package and marked on the SIM trace as "Rejected," with an explanation of the reasons for the rejection.

1.1.4 SIM traces generated during each GC column performance check analysis; peak profile outputs of the reference signal used to document the mass resolution.

1.1.5 Documentation of acceptable MS calibration (Section 8, Exhibit D, and Exhibit E) for each confirmatory analysis. As applicable, submit peak matching box settings and calculations for accurate mass assignments and any other related printouts. State, in ppm, the level of mass accuracy achieved (Section 8.2.2, Exhibit D).

1.1.6 A chronological list of all analyses performed (Form H-6). If more than one GC/MS system is used, a chronological list is required for each system. The list must provide the Data System File name, the EPA sample number, and (if appropriate) the contractor laboratory sample number for each sample, blank, concentration calibration solution, performance check solution, or other pertinent analytical data. This list shall specify date and time of beginning of analysis. All sample/blank analyses performed during a 12-hour period must be accompanied by two GC column performance check solution analyses, one preceding and one following the sample/blank analyses. If multiple shifts are used, the ending GC column performance check sample analysis from one 12-hour period shall serve as the beginning analysis for the next 12-hour period; see Exhibit D, Section 8, for system performance criteria. The same schedule applies to the mass resolution check analysis. See Section 8.2.2, Exhibit D.

1.1.7 Verification of recovery of TCDDs from cleanup columns (Section 11.3, Exhibit D, and Section 4.2.1.2.2, Exhibit E).

1.2 Sample extracts and unused sample portions -- Unused portions of samples and sample extracts shall be retained by the Contractor for a period of six months after receipt. When directed in writing by the Project Officer (PO) or Sample Management Office (SMO), the Contractor shall ship (not at Contractor's expense but in accordance with Department of Transportation Regulations) specific samples and/or extracts to specified locations and persons. After six months, upon obtaining PO or SMO clearance, remaining samples and extracts shall be disposed of by the Contractor at Contractor's expense, in accordance with applicable regulations concerning the disposal of such materials.

1.3 Document Control and Chain-of-Custody Package -- The Document Control and Chain-of-Custody Package includes all laboratory records received or generated for a specific case, that have not been previously submitted to EPA as a deliverable. These items include but are not limited to: sample tags, custody records, sample tracking records, analysts logbook pages, bench sheets, chromatographic charts, computer printouts, raw data summaries, instrument logbook pages, correspondence, and the document inventory (Exhibit G).

NOTE: Pages from logbooks or bench sheets kept exclusively in a high-hazard area (containment facility) need not be copied.

1.4 Monthly Sample Status Report -- The Monthly Sample Status Report shall provide the status of all samples the Contractor has received or has had in-house during the calendar month. Required status information includes: samples received, samples extracted, samples analyzed, samples rerun, and samples which required special cleanup. All samples shall be identified by the appropriate EPA sample, case and batch/shipment numbers.

1.5 Daily Sample Status Report -- In response to a verbal request from the Sample Management Office or the Project Officer, the Contractor must verbally provide sample status information on a same-day basis. Should written confirmation be requested, the Contractor must send the daily sample status information in a written form that same day using first-class mail service. The required Daily Sample Status information shall include the items noted for the Monthly Sample Status Report and, in addition, shall require information on sample analysis reports in progress and analysis reports submitted/mailed.

2. In accordance with applicable delivery requirements, the Contractor shall deliver specified items per the following Contract Reporting Schedule (Section 2.1). Recipients include the CLP Sample Management Office, the EMSL/LV QA Division, the appropriate Regional Technical Officer and NEIC.

2.1 Contract Reporting Schedule

CONTRACT REPORTING SCHEDULE

Item No.	Report	No. Copies	Delivery Schedule	Report Distribution			
				SMO	EMSL/LV	Region	NEIC
1	Sample Data Package	3	30 days after validated sample receipt date -OR-	X	X	X	
			10 days after initial data due date	X	X	X	
2	Sample Extracts		Within 180 days after analysis, 7 days after request by Project Officer or SMO	As directed			

(Continued)

1. SCOPE AND APPLICATION

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1.1.7 Verification of recovery of TCDDs from cleanup columns (Section 11.3, Exhibit D, and Section 4.2.1.2.2, Exhibit E).

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1.5 Daily Sample Status Report -- In response to a verbal request from the Sample Management Office or the Project Officer, the Contractor must verbally provide sample status information on a same-day basis. Should written confirmation be requested, the Contractor must send the daily sample status information in a written form that same day using first-class mail service. The required Daily Sample Status information shall include the items noted for the Monthly Sample Status Report and, in addition, shall require information on sample analysis reports in progress and analysis reports submitted/mailed.

2. In accordance with applicable delivery requirements, the Contractor shall deliver specified items per the following Contract Reporting Schedule (Section 2.1). Recipients include the CLP Sample Management Office, the EMSL/LV QA Division, the appropriate Regional Technical Officer and NEIC.

2.1 Contract Reporting Schedule

CONTRACT REPORTING SCHEDULE

Item No.	Report	No. Copies	Delivery Schedule	Report Distribution SMO	EMSL/LV	Region	NEIC
1	Sample Data Package	3	30 days after validated sample receipt date	X	X	X	
			-OR-				
			10 days after initial data due date	X	X	X	
2	Sample Extracts		Within 180 days after analysis, 7 days after request by Project Officer or SMO		As directed		

(Continued)

CONTRACT REPORTING SCHEDULE (Continued)

Item No.	Report	No. Copies	Delivery Schedule	Report Distribution			
				SMO	EMSL/LV	Region	NEIC
3	Document Control & Chain-of-Custody Package	1 Pkg	7 days after request by Project Officer or SMO	X			X
4	Monthly Sample Status Report	2	5 days following end of each calendar month	X	X		
5	Daily Sample Status Report	1	Verbal and/or written upon request by SMO or PO; maximum frequency is daily.		As directed		

NOTE: All results shall be reported total and complete.

2.2 Addresses for distribution

SMO	EMSL-LV	NEIC
CLP Sample Management Office P. O. Box 818 Alexandria, VA 22313	US EPA EMSL-LV QA Division Box 15027 Las Vegas, NV 89114 Attn: Data Audit Staff	US EPA NEIC Bldg. 53 Box 25227 Denver Fed. Center Denver, CO 80225
For overnight deliveries, use street address: 300 N. Lee St., Suite 200 Alexandria, VA 22314	For overnight deliveries, use street address: 944 E. Harmon Ave. Executive Center Las Vegas, NV 89109	

Regional Technical Officer -- Following contract award and prior to Contractor's receipt of the first batch of samples, the Sample Management Office will provide the Contractor with the list of Technical Officers for the ten EPA Regions. SMO will provide the Contractor with updated Regional address/name lists as necessary throughout the period of the contract.

3. FORM INSTRUCTION GUIDE

This section includes specific instructions for the completion of all required forms. These include instructions on header information as well as specific details to the bodies of individual forms. Instructions are arranged in the following order:

Data Summary (Form H-1)
Initial Calibration Summary (Form H-2; 2 pages)
Routine Calibration Summary (Form H-3)
GC and Mass Resolution Check Summary (Form H-4)
Quality Control Summary (Form H-5)
Chronological List of All Analyses Performed (Form H-6)
GC Operating Conditions (Form H-7)
HRMS TCDD Calibration Report Form (Form H-8)
High-Resolution MS TCDD Data Report Form (Form H-9)

3.1 Data Summary (Form H-1)-- This form is used for summarizing the results from all samples in the batch. The detailed results are available on Form H-8 for each sample.

Complete the header information at the top of the page, including laboratory name, case number and batch/shipment number (from the dioxin shipment record), and matrix (soil, sediment, water).

Complete the form using one horizontal row for each sample.

The SMO sample number should be suffixed with the appropriate letter code as needed.

The TCDD retention time should be reported in minutes and seconds.

TCDD levels are reported as parts per trillion (ppt) regardless of the matrix. Total TCDD concentration (in ppt) is the sum of the concentrations of all TCDDs reported on Form H-9.

The S/N criteria apply to m/z 259, 320, 322 (for unlabeled TCDD) and m/z 322 and 334 (internal and recovery standards). The symbols used are: (+) all S/N ratios are 2.5 or greater including all TCDDs present, (-) S/N ratio for native 2,3,7,8-TCDD, the internal or the recovery standard are less than 2.5, (0) other suspected TCDDs are present but did not meet the S/N criteria.

The file name is the HRGC/HRMS file name and is used for tracking results and raw data.

The comments column should be used for any remarks specific to a particular sample.

3.2 Initial Calibration Summary (Form H-2): Page 1 -- The header information should be filled in. The column headings are similar to those on Form H-1.

$$\mathrm{RRF(I)} = \frac{A_x \cdot Q_{IS}}{Q_x \cdot A_{IS}}$$

$$RRF(II) = \frac{A_{IS} \cdot Q_{RS}}{Q_{IS} \cdot A_{RS}}$$ (Section 8.3.4.5, Exhibit D)

Page 2 -- The header information should be filled in. For each RRF, the mean, percent relative standard deviation (%RSD) and number of runs (N) are reported; N must be at least three (3) for each HRCC solution. The grand means ($\overline{RRFs}$) are the mean of the individual means and are reported with their %RSD and N. The routine calibration relative response factor permissible ranges are also reported (Section 8.3.4.8, Exhibit D).

3.3 Routine Calibration Summary (Form H-3) -- The header information includes case and batch numbers in addition to the laboratory and instrument identification.

The columns are the same as on Page 1 of Form H-2. The results reported are for the routine calibration runs rather than the initial calibration. The calculated RRF(I) and RRF(II) must be within the routine calibration relative response factor permissible ranges (Section 8.3.4.8, Exhibit D) and other criteria listed in Section 8.6, Exhibit D must be met before further analysis is performed.

3.4 GC and Mass Resolution Check Summary (Form H-4) -- The header information should be filled in. The TCDD isomer resolution (% valley) is measured from the column performance check solution (Section 8.1.2, Exhibit D). The resolving power and mass measurement error are measured using PFK (or equivalent) (Section 8.2, Exhibit D).

3.5 Quality Control Summary (Form H-5) -- The items should be completed as indicated. The "other interferences" should be included even if they only occur at one mass.

Form H-5 in conjunction with Form H-9 is used to report results relative to the fortified field blank pair and rinsate analyses.

The total TCDD retention time window is a window that includes all of the TCDD isomers and is based on the first and last eluting isomers in the GC column performance check solution using the conditions summarized in Form H-7. All materials used should be recorded in the standard/reagent QC table. Standards provided by EPA should be listed, however, the QC columns may be left blank as these are reference materials.

3.6 Chronological List of All Analyses Performed (Form H-6) -- The header information should be filled in. If more than one instrument is used, use one form per instrument.

The "Analysis Identification" column should contain enough information for the data user to clearly identify the analysis, i.e., HRCC 2 Routine Calibration, Fortified Field Blank A, Fortified Field Blank B, Reanalysis of Sample #1, 2, 3, 4, etc. The "SMO #" column should be used only for samples etc. which have an assigned SMO sample number.

3.7 GC Operating Conditions (Form H-7) -- This form must be filled out to describe the GC operating conditions used to analyze a batch of samples and to analyze the GC performance evaluation check solution.

3.8 HRMS TCDD Calibration Report (Form H-8) -- This form is to be filled in for each initial and routine calibration analysis made. It will be the first page of the chromatograms and calculations for that analysis. It is suggested that this form be used as a worksheet for completing Forms H-2 and H-3. S/N ratios greater than five (5) may be reported with a (+); S/N ratios of five or less must have a numerical value reported with accompanying chromatograms scaled so that the measurements may be checked by the data user.

3.9 High-Resolution MS TCDD Data Report (Form H-9) -- This form contains the details of the data reported in summary on Form H-1. It will be the first page of the chromatograms and calculations for each sample including the fortified field blank pair samples. All data presented (retention times, areas, and S/N ratios) must also be available on the accompanying chromatograms. The chromatograms must be scaled so that the data user may check any S/N ratios that are near or below five (5). It is suggested that this form be used as a worksheet for completing Form H-1.

4. REPORTING REQUIREMENTS SUMMARY:

Items that must be included with the data package:

4.1 Complete identification of the samples analyzed (sample numbers and type).

4.2 The dates and times at which all analyses were accomplished. This information should also appear on each selected ion current profile included with the report.

4.3 Raw mass chromatographic data which consist of the absolute peak heights or peak areas of the signals observed for the ion masses monitored.

4.4 The calculated ratios of the intensities of the $M^{+\circ}$ to $(M+2)^{+\circ}$ molecular ions for all TCDD isomers detected.

4.5 The calculated concentrations of native 2,3,7,8-TCDD and other TCDD isomers for each sample analyzed, expressed in picograms TCDD per gram of sample (that is, parts per trillion), as determined from the raw data. If no TCDDs are detected, the notation "Not Detected" or "N.D." is used, and the minimum detectable concentrations (or detection limits) are reported.

HIGH RESOLUTION
FORM H-1 DATA SUMMARY
HRGC/HRMS DIOXIN ANALYSIS

Lab: ______________ Case # ______________ Batch/Shipment # ______________ Matrix: ______________

SMO Sample Number	TCDD Retention Time		ppt 2,3,7,8-TCDD		ppt Total TCDD	Relative Ion Abundance Ratios			%Rec. (IS)	S/N Criteria	Inst. ID	Analysis Date	Time	File Name	Comments
	2,3,7,8	(IS)	Meas.	DL		$\frac{320}{322}$	$\frac{332}{334}$(IS)	$\frac{332}{334}$(RS)							

RB - Reagent Blank
N - Unlabeled TCDD Spike
D - Duplicate
FB - Field Blank
SR - Sample Rerun
ER - Extract Reanalysis

ND - Not Detected
DL - Detection Limit
MB - Method Blank
Rec - Recovery

Matrix: S - Soil
W - Water
O - Other

S/N Criteria: report (+) if all S/N > 2.5
report (—) if 2,3,7,8-TCDD,
$^{13}C_{12}$-2,3,7,8-TCDD or
$^{13}C_{12}$-1,2,3,4-TCDD S/N < 2.5
report (0) if other TCDDs are suspected not to meet criteria

HIGH RESOLUTION
FORM H-2 INITIAL CALIBRATION SUMMARY

page 1 of 2

Lab: ______________________ **Contract #:** ______________________ **Instrument ID:** ______________________

Calibration Standard	File Name	Date	Time	m/z 320/322[a]	m/z 332/334(IS)[a]	m/z 332/334(RS)[a]	S/N Criteria	RRF(I)[b]	RRF(II)[c]	Comments

[a] Ion ratios must be in the range of 0.67 to 0.90

[b] 2,3,7,8-TCDD versus $^{13}C_{12}$-2,3,7,8-TCDD

[c] $^{13}C_{12}$-2,3,7,8-TCDD versus $^{13}C_{12}$-1,2,3,4-TCDD

S/N Criteria: report (+) if greater than 2.5
report (-) if less than 2.5

HIGH RESOLUTION
FORM H-2 INITIAL CALIBRATION SUMMARY

page 2 of 2

Lab: ____________ Contract #: ____________ Instrument #: ____________

Date of Intitial Calibration: ____________

	RRF (I) Mean	% RSD	N	RRF (II) Mean	% RSD	N
HRCC1						
HRCC2						
HRCC3						
HRCC4						

$\overline{\text{RRF (I)}}$ Grand Mean: ____________

% RSD: ____________

N: ____________

$\overline{\text{RRF (II)}}$ Grand Mean: ____________

% RSD: ____________

N: ____________

Routine Calibration Permissible Range: ____________
RRF (I) = 2,3,7,8-TCDD vs
$^{13}C_{12}$-2,3,7,8-TCDD

Routine Calibration Permissible Range: ____________
RRF (II) = $^{13}C_{12}$-2,3,7,8-TCDD vs
$^{13}C_{12}$-1,2,3,4-TCDD

HIGH RESOLUTION
FORM H-3 ROUTINE CALIBRATION SUMMARY

Lab: ____________ **Case #:** ____________ **Batch #:** ____________ **Instrument ID:** ____________

Calibration Standard	File Name	Date	Time	m/z 320/322[a]	m/z 332/334(IS)[a]	m/z 332/334(RS)[a]	S/N Criteria	RRF(I)[b]	RRF(II)[c]	Comments

[a] Ion ratios must be in the range of 0.67 to 0.90

[b] 2,3,7,8-TCDD versus $^{13}C_{12}$-2,3,7,8-TCDD

[c] $^{13}C_{12}$-2,3,7,8-TCDD versus $^{13}C_{12}$-1,2,3,4-TCDD

S/N Criteria: report (+) if greater than 2.5
report (-) if less than 2.5

HIGH RESOLUTION
FORM H-4 GC AND MASS RESOLUTION CHECK SUMMARY

Lab: ________________ **Case #** ________________ **Batch #** ________________

Date	Inst. ID	Sol. ID	Time	File Name	TCDD Isomer Resolution (% Valley)	Resolving Power at 10% Valley	*Mass Measurement Error (PPM)

*Mass used for mass measurement error calculation ________________________

HIGH RESOLUTION
FORM H-5 QUALITY CONTROL SUMMARY

Lab: ____________ **Case #** ____________ **Batch #** ____________

Number of samples in batch: ____________

Mean % of recovery for the I.S.: ____________ **# of data points:** ____________

Fortified field blank A, % recovery ($^{13}C_{12}$-2,3,7,8-TCDD): ____________ **SMO Sample #:** ____________

	No	Yes		
Contamination by 1,2,3,4-TCDD	☐	☐	____________	Estimated Concentration (ppt)
$^{13}C_{12}$-1,2,3,4-TCDD	☐	☐	____________	

Other interferences: Retention times: ____________

Estimated concentrations (ppt): ____________

Fortified field blank B, % recovery ($^{13}C_{12}$-1,2,3,4-TCDD): ____________ **SMO Sample #:** ____________

	No	Yes		
Contamination by 2,3,7,8-TCDD	☐	☐	____________	Estimated Concentration (ppt)
$^{13}C_{12}$-2,3,7,8-TCDD	☐	☐	____________	

Other interferences: Retention times: ____________

Estimated concentrations (ppt): ____________

Rinsate, % recovery: ____________ **SMO sample #:** ____________

	No	Yes		
Contamination by 2,3,7,8-TCDD	☐	☐	____________	Estimated Concentration (pg/mL)
Other TCDD	☐	☐	____________	

Duplicate analysis. SMO sample #: ____________ $^{13}C_{12}$-2,3,7,8-TCDD Mean Recovery: ____________

Percent Relative difference $^{13}C_{12}$-2,3,7,8-TCDD (Recovery) ____________

Percent relative difference $^{13}C_{12}$-2,3,7,8-TCDD (Concentration) ____________

Percent relative difference Total TCDD (Concentration) ____________

Method blank file name: ____________ **Total TCDD retention time window from column performance check:** ____________

Standard/Reagent Type	HRMS Lab. Number or Mfg. #	Origin	Date of QC	QC File Name	Results of QC

Continue as needed

HIGH RESOLUTION
FORM H-6 CHRONOLOGICAL LIST OF ALL ANALYSES PERFORMED

Lab: ______________________ **Case #** ____________________ **Batch #** ____________________

Instrument ID: ____

File Name	Analysis Identification	SMO Number	Date	Time

HIGH RESOLUTION
FORM H-7 GC OPERATING CONDITIONS

Lab: ______________________ **Instrument ID:** ______________________

GC Column: ______________________

Film Thickness: ______________________

Column Dimensions: ______________________

Initial Column Temperature: ______________________

Temperature Program: ______________________

Injector Temperature: ______________________

Interface Temperature: ______________________

Injection Mode: ______________________

Injection Volume: ______________________

Splitless Valve Closed Time: ______________________

Septum Purge Flow: ______________________

Injector Sweep Flow: ______________________

Carrier Gas Flow Rate (ml/min or cm/sec): ______________________

HIGH RESOLUTION
FORM H-8 HRMS TCDD CALIBRATION REPORT FORM

Lab: **Calibration Solution:**

Case #: **GC Column:**

Batch/Shipment #: **Date of Initial Calibration:**

Instrument ID: **Analysis Date:** **Time:**

Calibration: **File Name**
Initial
Routine

2,3,7,8-TCDD	**Retention Time**	**Area**	**Ratios**	**S/N**[a]
m/z 258.930				
319.897			$\frac{320}{322}$	
321.894				
$^{13}C_{12}$-2,3,7,8-TCDD				
m/z 331.937			$\frac{332}{334}$	
333.934				
$^{13}C_{12}$-1,2,3,4-TCDD				
m/z 331.937			$\frac{332}{334}$	
m/z 333.934				

[a] If S/N is greater than 5, enter (+); if less than 5, enter the measured ratio

HIGH RESOLUTION
FORM H-9 HIGH RESOLUTION MS TCDD DATA REPORT FORM

Lab: ______ **SMO Sample #:** ______
Case #: ______ **File Name:** ______
Batch/Shipment #: ______ **Matrix: Water** ______ **Soil** ______ **Other** ______
Instrument ID: ______ **Analysis Date:** ______ **Time:** ______
GC Column ______
Aliquot Wt. or Vol. ______ **Circle One** g L **Percent Moisture** ______ **Extraction Date:** ______
Measured ppt 2,3,7,8-TCDD: ______ **Detection Limit 2,3,7,8-TCDD:** ______
Estimated Total TCDD (ppt): ______ **Report Date:** ______

2,3,7,8-TCDD	Retention Time	Area	Ratios	S/N*
m/z 258.930				
319.897			$\frac{320}{322}$	
321.894				
$^{13}C_{12}$-2,3,7,8-TCDD				
m/z 331.937			$\frac{332}{334}$	
333.934				
$^{13}C_{12}$-1,2,3,4-TCDD				
m/z 331.937			$\frac{332}{334}$	
m/z 333.934				

Percent Recovery $^{13}C_{12}$-2,3,7,8-TCDD: ______

Other TCDDs

Retention Time	$\frac{320}{322}$	S/N* 259	S/N* 320	S/N* 322	Estimated Concentration (ppt)

*If S/N is greater than 5, enter (+); if less than 5 enter the measured ratio

4.6 The same raw and calculated data which are provided for the actual samples will also be reported for the duplicate analyses, the method blank analyses, the fortified field blank pair and rinsate analyses, and any other QA or performance sample analyzed in conjunction with the actual sample set(s).

4.7 The recoveries of the internal standard ($^{13}C_{12}$-2,3,7,8-TCDD) in percent.

4.8 The calibration data, including relative response factors calculated from the calibration procedure described in Section 8.3, Exhibit D. Data showing that these factors have been verified at least once during each 12-hour period of operation must be included (Section 8.5, Exhibit D). Exact mass measurement error. Include peak matching box settings and calculations as appropriate.

4.9 The calculated dry weight of the original soil or sediment sample portion based on the dry weight determination of another sample portion of approximately equal wet weight. The exact volumes of the water and rinsate samples analyzed.

4.10 Documentation of the source of all TCDD standards used and available specifications on purity.

4.11 In addition, each report of analyses will include the following selcted ion current profiles: 1) those obtained from all samples analyzed, 2) those from each GC column performance check, and 3) those from the calibration solutions. The peak profile from each mass resolution check must also be part of the data package.

4.12 Identify which HRGC/HRMS system was used for the analyses (manufacturer and laboratory identification number of system - 01, 02, 03, etc.).

4.13 GC operating conditions such as type of GC column, film thickness, column dimensions, initial column temperature, temperature program, injector temperature, interface temperature, injection mode and volume, valve time (valve flush), septum purge flow, flow rate, and total injector flow should be provided (Form H-7).

EXHIBIT C

Sample Rerun Requirements

1. SCOPE AND APPLICATION

The Contractor shall be required to reextract and/or perform additional cleanup and reanalyze certain samples or batches of samples in a variety of situations that may occur in the process of contract performance. (For purposes of this contract, the term "sample rerun" shall indicate sample extraction of a fresh 10-g soil or sediment portion or 1-L aqueous sample, followed by cleanup and analysis, and the term "extract reanalysis" shall indicate analysis of another aliquot of the final extract.

In situations where the sample rerun is required due to matrix effects, interferences or other problems encountered because of very complex samples, the Government will pay the Contractor for the sample reruns. Such sample reruns shall be billable and accountable under the specified contract allotment of automatic reruns.

In situations where the sample rerun or the extract reanalysis is required due to Contractor materials, equipment or instrumentation problems, or lack of contractor's adherence to specified contract procedures, the sample rerun or extract reanalysis shall not be billable under the terms of the contract.

Contractor's failure to perform any of the sample reruns or extract reanalyses specified herein, either billable or non-billable, shall be construed as Contractor nonperformance and may result in termination of the contract for default by the Contractor.

2. Required Sample Reruns and Extract Reanalyses

2.1 Automatic sample reruns and extract reanalyses that may be billable as such under the contract.

2.1.1 If the percent recovery for the internal standard $^{13}C_{12}$-2,3,7,8-TCDD was outside of the acceptance limits of $\geq$40 percent and $\leq$120 percent, the Contractor shall reextract and reanalyze the sample. If the percent recovery for the sample rerun is still outisde the acceptance limits, then both analyses can be billed if the recoveries from both analyses are either <40% or >120%. If, however, the percent recovery for the sample rerun is within the acceptance limits, or if it is still outside the acceptance limits but the percent recoveries from the original analysis and the sample rerun are not both either <40% or >120%, then the sample rerun may not be billed.

2.1.2 If the internal standard was not found upon monitoring m/z 331.937 and 333.934, the Contractor shall reextract and reanalyze the sample. If the internal standard is not found in the sample rerun, the sample rerun is billable. If the internal standard is found in the sample rerun, then the sample rerun is not billable.

2.1.3 If either one of the isotope abundance ratios for m/z 319.897/321.894 or for 331.937/333.934 is less than 0.67 or greater than 0.90 and all other criteria contained in Section 12.4 of Exhibit D are met, then the extract shall be reanalyzed. If both ion abundance ratios now meet the criterion, these values shall be reported as the isotope abundance ratios, and the Contractor shall not bill the Government for the extract reanalysis. If the ratio in question is still outside the criterion, the Contractor shall rerun the sample (Section 7.2, Exhibit E). If either one of the ratios determined from the sample rerun is still outside the acceptance limits, then both runs and the extract reanalysis can be billed if the corresponding isotope abundance ratios from both runs are either <0.67 or >0.90. If, however, both isotope abundance ratios from the sample rerun meet the criteria, or if both corresponding isotope abundance ratios from the original run and the sample rerun are not both either <0.67 or >0.90, then the extract reanalysis and the sample rerun may not be billed.

2.1.4 If the recoveries of 2,3,7,8-TCDD (Section 4.2.1.1.3.1, Exhibit E) and/or 1,2,3,4-TCDD (Section 4.2.1.2, Exhibit E) in the fortified field blank pair are <40% or >120%, the Contractor shall reextract and reanalyze a second portion of the field blank sample (Section 4.2, Exhibit E). If the percent recoveries for the sample rerun are still outside the acceptance limits, then both analyses can be billed as long as the recoveries from both analyses are either <40% or >120%. If, however, the percent recoveries for the sample rerun are within the acceptance limits, or if they are still outside the acceptance limits but the percent recoveries from the original run and the sample rerun are not both either <40% or >120%, then the sample rerun may not be billed.

NOTE: Fortified field blanks as described in Sections 4.2.1.1.4 and 4.2.1.2.2, Exhibit E, can never be billed.

2.2 Automatic sample extract dilution and HRGC/HRMS analysis, billable as such under the Contract.

If any individual or group of coeluting TCDD isomer concentrations in the 10-uL final extract exceeds 100 pg/uL, the analyst will perform a dilution as specified in Section 13.3, Exhibit D, and reanalyze the diluted portion using HRGC/HRMS.

2.3 Sample reruns and/or extract reanalyses to be performed at Contractor's expense (i.e., not billable under the terms of the contract).

2.3.1 If the method blank contains any signal in the TCDD retention time window at or above the method quantitation limit (2 ppt for soil and sediment and 20 ppq for aqueous samples), the Contractor shall rerun all positive samples in the batch of samples (Section 4.1.2, Exhibit E).

2.3.2 If the system performance using the GC column performance check (PC) solution does not meet specified criteria, the Contractor shall take corrective action, demonstrate acceptable GC column performance, and reanalyze the extracts from all positive samples run during the time period between the last acceptable PC run and the unacceptable PC run (Section 2.4, Exhibit E).

2.3.3 If a false positive is reported for an uncontaminated soil (blind QC) sample, upon notification by the Sample Management Office the Contractor shall reextract and reanalyze all samples reported as positive in the associated batch of samples (Section 8.1.1, Exhibit E).

2.3.4 If the analysis results for a performance evaluation blind QC sample fall outside of EPA-established acceptance windows, upon notification of the Sample Management Office the Contractor shall reextract and reanalyze the entire associated batch of samples (Section 8.4.1, Exhibit E).

2.3.5 If the isotope abundance ratio for m/z 319.897/321.894 or for 331.937/333.934 is less than 0.67 or greater than 0.90, and all other criteria contained in Section 12.4 of Exhibit D are met, then the extract shall be reanalyzed. If the ion abundance ratio in question now meets the criterion, this value shall be reported as the isotope abundance ratio, and the Contractor shall not bill the Government for the extract reanalysis.

2.3.6 If the system performance mass resolution check does not meet the specified criterion, the Contractor shall take corrective action, demonstrate acceptable mass resolution and reanalyze the extract from all positive samples analyzed during the time period between the last acceptable mass resolution check and the unacceptable mass resolution check (Section 2.4, Exhibit E).

EXHIBIT D

Analytical Method

2,3,7,8-Tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) and Total TCDDs in Soil/Sediment and Water by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry

EXHIBIT D

Section	Subject	Page
1	Scope and Application.	D-1
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1. SCOPE AND APPLICATION

1.1 This method provides procedures for the detection and quantitative measurement of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD; CAS Registry Number 1746-01-6; Storet number 3475) at concentrations of 2 pg/g (2 parts per trillion) to 100 pg/g (100 parts per trillion) in 10-g portions of soil and sediment and at 20 pg/L (20 parts per quadrillion) to 1000 pg/L (1 part per trillion) in 1-L samples of water. Dilution of an aliquot of the final extract permits measurement of concentrations up to 1.2 ng/g (1.2 parts per billion) or 12 ng/L (12 parts per trillion), respectively. This method also allows the estimation of quantities of total TCDD present in the sample. Samples containing concentrations of any individual TCDD isomer or group of coeluting TCDD isomers greater than 1.2 ng/g or 12 ng/L must be analyzed by a protocol designed for such concentration levels, with an appropriate instrument calibration range.

1.2 The minimum measurable concentration is estimated to be 2 pg/g (2 parts per trillion) for soil and sediment samples and 20 pg/L (20 parts per quadrillion) for water samples, but this depends on kinds and concentrations of interfering compounds in the sample matrix.

1.3 This method is designed for use by analysts who are experienced in the use of high-resolution gas chromatography/high-resolution mass spectrometry.

CAUTION: TCDDs are assumed to be extremely hazardous. It is the laboratory's responsibility to ensure that safe handling procedures are employed.

2. SUMMARY OF METHOD

One thousand pg of $^{13}C_{12}$-2,3,7,8-TCDD (internal standard) are added to a 10-g portion of a soil/sediment sample (weighed to 3 significant figures) or a 1-L aqueous sample, and the sample is extracted with 200 to 250 mL benzene using a Soxhlet apparatus for soils and sediments with a minimum of 3 cycles per hour, or with methylene chloride using a continuous liquid-liquid extractor for aqueous samples for 24 hours. A separatory funnel and 3 x 60 mL methylene chloride may also be used for aqueous samples. After appropriate cleanup, 10 uL of a tridecane solution of the recovery standard ($^{13}C_{12}$-1,2,3,4-TCDD) are added to the extract which is then concentrated to a final volume of 10 uL. One to three uL of the concentrated extract is injected into a gas chromatograph with a capillary column interfaced to a high-resolution mass spectrometer capable of rapid multiple ion monitoring at resolutions of at least 10,000 (10 percent valley).

Identification of 2,3,7,8-TCDD is based on the detection of the ions m/z 319.897 and 321.894 at the same GC retention time and within -1 to +3 seconds GC retention time of the internal standard masses of m/z 331.937 and 333.934. Confirmation of 2,3,7,8-TCDD (and of other TCDD isomers) is based on the ion m/z 258.930 which results from loss of COCL by the parent molecular ion.

3. DEFINITIONS

3.1 Concentration calibration solutions -- solutions containing known amounts of the analyte (unlabeled 2,3,7,8-TCDD), the internal standard $^{13}C_{12}$-2,3,7,8-TCDD and the recovery standard $^{13}C_{12}$-1,2,3,4-TCDD; they are used to determine instrument response of the analyte relative to the internal standard and of the internal standard relative to the recovery standard.

3.2 Field blank -- a portion of soil/sediment or water uncontaminated with 2,3,7,8-TCDD and/or other TCDDs.

3.3 Rinsate -- a portion of solvent used to rinse sampling equipment; the rinsate is analyzed to demonstrate that samples have not been contaminated during sampling.

3.4 Internal standard -- $^{13}C_{12}$-2,3,7,8-TCDD, which is added to every sample (except the blank described in Sections 4.2.1 of Exhibit E) and is present at the same concentration in every method blank and quality control sample. It is added to the soil/sediment or aqueous sample before extraction and is used to measure the concentration of each analyte. Its concentration is measured in every sample, and percent recovery is determined using an internal standard method.

3.5 Recovery standard -- $^{13}C_{12}$-1,2,3,4-TCDD which is added to every sample extract (except for the blank discussed in Sections 4.2.1, Exhibit E) just before the final concentration step and HRGC-HRMS analysis.

3.6 Laboratory method blank -- this blank is prepared in the laboratory through performing all analytical procedures except addition of a sample aliquot to the extraction vessel.

3.7 GC column performance check mixture -- a mixture containing known amounts of selected standards; it is used to demonstrate continued acceptable performance of the capillary column, i.e., separation ($\leq$ 25% valley) of 2,3,7,8-TCDD isomer from all other 21 TCDD isomers, and to define the TCDD retention time window.

3.8 Performance evaluation sample -- a soil, sediment or aqueous sample containing a known amount of unlabeled 2,3,7,8-TCDD and/or other TCDDs. It is distributed by the EMSL-LV to potential contractor laboratories who must analyze it and obtain acceptable results before being awarded a contract for sample analyses (see IFB Pre-Award Bid Confirmations). It may also be included as an unspecified ("blind") QC sample in any sample batch submitted to a laboratory for analysis.

3.9 Relative response factor -- response of the mass spectrometer to a known amount of an analyte relative to a known amount of an internal standard.

3.10 Mass resolution check -- standard method used to demonstrate static resolution of 10,000 minimum (10% valley definition).

3.11 Positive response for a blank -- defined as a signal in the TCDD retention time window, at any of the masses monitored, which is equivalent to or above the method quantitation limit (2 ppt for soil and sediment, and 20 ppq for aqueous samples).

3.12 Sample rerun -- extraction of another 10-g soil or sediment sample portion or 1-L aqueous sample, followed by extract cleanup and extract analysis.

3.13 Extract reanalysis -- analysis of another aliquot of th final extract.

4. INTERFERENCES

Chemicals which elute from the GC column within ±10 scans of the internal and/or recovery standard (m/z 331.937 and 333.934) and which produce within the TCDD retention time window ions at any of the masses used to detect or quantify TCDD are potential interferences. Most frequently encountered potential interferences are other sample components that are extracted along with TCDD, e.g. PCBs, chlorinated methoxybiphenyls, chlorinated hydroxydiphenylethers, chlorinated benzylphenylethers, chlorinated naphthalenes, DDE, DDT, etc. The actual incidence of interference by these chemicals depends also upon relative concentrations, mass spectrometric resolution, and chromatographic conditions. Because very low levels of TCDDs must be measured, the elimination of interferences is essential. High-purity reagents and solvents must be used and all equipment must be scrupulously cleaned. Blanks (Exhibit E, Quality Control, Section 4) must be analyzed to demonstrate absence of contamination that would interfere with TCDD measurement. Column chromatographic procedures are used to remove some coextracted sample components; these procedures must be performed carefully to minimize loss of TCDDs during attempts to increase their concentration relative to other sample components.

5. SAFETY

The toxicity or carcinogenicity of each reagent used in this method has not been precisely defined; however, each chemical compound should be treated as a potential health hazard. From this viewpoint, exposure to these chemicals must be reduced to the lowest possible level by whatever means available. The laboratory is responsible for maintaining a file of current OSHA regulations regarding the safe handling of the chemicals specified in this method. A reference file of material data handling sheets should also be made available to all personnel involved in the chemical analysis. Additional references to laboratory safety are identified (1-3) (page D-21). 2,3,7,8-TCDD has been identified as a suspected human or mammalian carcinogen. The laboratory is responsible for ensuring that safe handling procedures are followed.

6. APPARATUS AND EQUIPMENT

6.1 High-Resolution Gas Chromatograph/High-Resolution Mass Spectrometer/Data System (HRGC/HRMS/DS)

6.1.1 The GC must be equipped for temperature programming, and all required accessories must be available, such as syringes, gases, and a capillary column. The GC injection port must be designed for capillary columns. The use of splitless injection techniques is recommended. On-column injection techiques can be used but this may severely reduce column lifetime for nonchemically bonded columns. When using the method in this protocol, a 2-uL injection volume is used consistently. With some GC injection ports, however, 1-uL injections may produce improved precision and chromatographic separation. A 1- to 3-uL injection volume may be used if adequate sensitivity and precision can be achieved.

NOTE: If 1 uL or 3 uL is used at all as injection volume, the injection volumes for all extracts, blanks, calibration solutions and the performance check sample must be 1 uL or 3 uL.

6.1.2 Gas Chromatograph-Mass Spectrometer Interface

The GC-MS interface may include enrichment devices, such as a glass jet separator or a silicone membrane separator, or the gas chromatograph can be directly coupled to the mass spectrometer ion source. The interface may include a diverter valve for shunting the column effluent and isolating the mass spectrometer ion source. All components of the interface should be glass or glass-lined stainless steel. The interface components should be compatible with 300°C temperatures. The GC/MS interface must be appropriately designed so that the separation of 2,3,7,8-TCDD from the other TCDD isomers which is achieved in the gas chromatographic column is not appreciably degraded. Cold spots and/or active surfaces (adsorption sites) in the GC/MS interface can cause peak tailing and peak broadening. It is recommended that the GC column be fitted directly into the MS ion source. Graphite ferrules should be avoided in the GC injection port since they may adsorb TCDD. Vespel™ or equivalent ferrules are recommended.

6.1.3 Mass Spectrometer

The static resolution of the instrument must be maintained at a minimum 10,000 (10 percent valley). The mass spectrometer must be operated in a selected ion monitoring (SIM) mode with total cycle time (including voltage reset time) of one second or less (Section 8.3.4.1). At a minimum, the following ions which occur at these masses must be monitored: m/z 258.930, 319.897, 321.894, 331.937 and 333.934.

6.1.4 Data System

A dedicated hardware or data system is employed to control the rapid multiple ion monitoring process and to acquire the data. Quantification data (peak areas or peak heights) and SIM traces (displays of intensities of each m/z being monitored as a function of time) must be acquired during the analyses. Quantifications may be reported based upon computer-generated peak areas or upon measured peak heights (chart recording).

NOTE: Detector zero setting must allow peak-to-peak measurement of the noise on the base line.

6.2 GC Columns

For isomer-specific determinations of 2,3,7,8-TCDD, the following fused silica capillary columns are recommended: a 60-m SP-2330 (SP-2331) column and a 50-m CP-Sil 88 column. However, any capillary column which separates 2,3,7,8-TCDD from all other TCDDs may be used for such analyses, but this separation must be demonstrated and documented. Minimum acceptance criteria must be determined per Section 8.1. At the beginning of each 12-hour period (after mass resolution has been demonstrated) during which sample extracts or concentration calibration solutions will be analyzed, column operating conditions must be attained for the required separation on the column to be used for samples. Operating conditions known to produce acceptable results with the recommended columns are shown in Table 2 at the end of this Exhibit.

6.3 Miscellaneous Equipment

6.3.1 Nitrogen evaporation apparatus with variable flow rate.

6.3.2 Balance capable of accurately weighing to ±0.01 g.

6.3.3 Centrifuge capable of operating at 2,000 rpm.

6.3.4 Water bath -- equipped with concentric ring cover and capable of being temperature-controlled within ±2°C.

6.3.5 Stainless steel spatulas or spoons.

6.3.6 Stainless steel (or glass) pan large enough to hold contents of 1-pint sample containers.

6.3.7 Glove box.

6.3.8 Drying oven.

6.4 Glassware

6.4.1 Soxhlet apparatus -- all-glass, Kontes 6730-02 or equivalent;

90 mm x 35 mm glass thimble; 500-mL flask; condenser of appropriate size.

6.4.2 Kuderna-Danish apparatus -- 500-mL evaporating flask, 10-mL graduated concentrator tubes with ground-glass stoppers, and 3-ball macro Snyder column (Kontes K-570001-0500, K-503000-0121 and K-569001-0219 or equivalent).

6.4.3 Mini-vials -- 1-mL borosilicate glass with conical-shaped reservoir and screw caps lined with Teflon-faced silicone disks.

6.4.4 Funnels -- glass; appropriate size to accommodate filter paper used to filter jar extract (volume of approximately 170 mL).

6.4.5 Separatory funnel -- 2000 mL with Teflon stopcock.

6.4.6 Continuous liquid-liquid extractors equipped with Teflon or glass connecting joints and stopcocks requiring no lubrication (Hershberg-Wolf Extractor - Ace Glass Company, Vineland, NJ; P/N 6841-10 or equivalent).

6.4.7 Chromatographic columns for the silica and alumina chromatography -- 1 cm ID x 10 cm long and 1 cm ID x 30 cm long.

6.4.8 Chromatographic column for the Carbopak cleanup -- disposable 5-mL graduated glass pipets, 6 to 7 mm ID.

6.4.9 Desiccator.

6.4.10 Glass rods.

NOTE: Reuse of glassware should be minimized to avoid the risk of cross contamination. All glassware that is reused must be scrupulously cleaned as soon as possible after use, applying the following procedure.

Rinse glassware with the last solvent used in it then with high-purity acetone and hexane. Wash with hot water containing detergent. Rinse with copious amounts of tap water and several portions of distilled water. Drain, dry and heat in a muffle furnace at 400°C for 15 to 30 minutes. Volumetric glassware must not be heated in a muffle furnace, and some thermally stable materials (such as PCBs) may not be removed by heating in a muffle furnace. In these two cases, rinsing with high-purity acetone and hexane may be substituted for muffle-furnace heating. After the glassware is dry and cool, rinse with hexane, and store inverted or capped with solvent-rinsed aluminum foil in a clean environment.

7. REAGENTS AND STANDARD SOLUTIONS

7.1 Column Chromatography Reagents

7.1.1 Alumina, acidic -- extract the alumina in a Soxhlet with methylene chloride for 6 hours (minimum of 3 cycles per hour) and activate it by heating in a foil-covered glass container for 24 hours at 190°C.

7.1.2 Silica gel -- high-purity grade, type 60, 70-230 mesh; extract the silica gel in a Soxhlet with methylene chloride for 6 hours (minimum of 3 cycles per hour) and activate it by heating in a foil-covered glass container for 24 hours at 130°C.

7.1.3 Silica gel impregnated with 40 percent (by weight) sulfuric acid -- add two parts (by weight) concentrated sulfuric acid to three parts (by weight) silica gel (extracted and activated), mix with a glass rod until free of lumps, and store in a screw-capped glass bottle.

7.1.4 Sulfuric acid, concentrated -- ACS grade, specific gravity 1.84.

7.1.5 Graphitized carbon black (Carbopack C or equivalent), surface of approximately 12 m^2/g, 80/100 mesh -- mix thoroughly 3.6 grams Carbopak C and 16.4 grams Celite 545® in a 40-mL vial. Activate at 130°C for six hours. Store in a desiccator.

7.1.6 Celite 545®, reagent grade, or equivalent.

7.2 Membrane filters or filter paper with pore size of $\leq$25 um; rinse with hexane before use.

7.3 Glass wool, silanized -- extract with methylene chloride and hexane and air-dry before use.

7.4 Desiccating Agents

7.4.1 Sodium sulfate -- granular, anhydrous; before use, extract it with methylene chloride for 6 hours (minimum of 3 cycles per hour) and dry it for $\geq$4 hours in a shallow tray placed in an oven at 120°C. Let it cool in a desiccator.

7.4.2 Potassium carbonate--anhydrous, granular; use as such.

7.5 Solvents -- high purity, distilled in glass: methylene chloride, toluene, benzene, cyclohexane, methanol, acetone, hexane; reagent grade: tridecane.

7.6 Concentration calibration solutions (Table 1) -- four tridecane solutions containing $^{13}C_{12}$-1,2,3,4-TCDD (recovery standard) and unlabeled 2,3,7,8-TCDD at varying concentrations, and $^{13}C_{12}$-2,3,7,8-TCDD (internal standard, CAS RN 80494-19-5) at a constant concentration must be used to calibrate the instrument. These concentration calibration solutions must be obtained from the Quality Assurance Division, US EPA, Environmental Monitoring Systems Laboratory (EMSL-LV), Las Vegas, Nevada. However, additional secondary standards may be obtained from commercial sources, and solutions may be prepared in the contractor laboratory. Traceability of standards must be verified against EPAsupplied standard solutions. Such procedures will be documented by laboratory SOPs as required in IFB Pre-award Bid Confirmations, part 2.f.(4). It is the responsibility of the laboratory to ascertain that the calibration solutions received are indeed at the appropriate concentrations before they are injected into the instrument.

NOTE: Serious overloading of the instrument may occur if the concentration calibration solutions intended for a low-resolution MS are injected into the high-resolution MS.

7.6.1 The four concentration calibration solutions contain unlabeled 2,3,7,8-TCDD and labeled $^{13}C_{12}$-1,2,3,4-TCDD at nominal concentrations of 2.0, 10.0, 50.0, and 100 pg/uL, respectively, and labeled $^{13}C_{12}$-2,3,7,8-TCDD at a constant nominal concentration of 10.0 pg/uL.

7.6.2 Store the concentration calibration solutions in 1-mL minivials at 4°C.

7.7 Column performance check mixture -- this solventless mixture must be obtained from the Quality Assurance Division, Environmental Monitoring Systems Laboratory, Las Vegas, Nevada, and dissolved by the Contractor in 1 mL tridecane. This solution will then contain the following components [including TCDDs (A) eluting closely to 2,3,7,8-TCDD, and the first- (F) and last-eluting (L) TCDDs when using the columns recommended in Section 6.2] at a concentration of 10 pg/uL of each of these isomers:

Analyte	Approximate Amount Per Ampule
Unlabeled 2,3,7,8-TCDD	10 ng
$^{13}C_{12}$-2,3,7,8-TCDD	10 ng
1,2,3,4-TCDD (A)	10 ng
1,4,7,8-TCDD (A)	10 ng
1,2,3,7-TCDD (A)	10 ng
1,2,3,8-TCDD (A)	10 ng
1,3,6,8-TCDD (F)	10 ng
1,2,8,9-TCDD (L)	10 ng

7.8 Sample fortification solution -- an isooctane solution containing the internal standard at a nominal concentration of 10 pg/uL.

7.9 Recovery standard spiking solution -- a tridecane solution containing the recovery standard at a nominal concentration of 10 pg/uL. Ten uL of this solution will be spiked into each sample extract (except for the fortified field blank A) before the final concentration step and HRGC/HRMS analysis. It is also used for the dilution of the extracts from samples with high TCDD levels (Section 13.3, Exhibit D).

7.10 Internal standard spiking solution -- a tridecane solution containing the internal standard ($^{13}C_{12}$-2,3,7,8-TCDD) at a nominal concentration of 10 pg/uL. Ten uL of this solution will be added to a fortified field blank extract (Section 4.2.1.1, Exhibit E). This is the only case where $^{13}C_{12}$2,3,7,8-TCDD is used for recovery purposes.

7.11 Field blank fortification solutions -- isooctane solutions containing the following TCDD isomers:

Solution A: 10.0 pg/uL of unlabeled 2,3,7,8-TCDD
Solution B: 10.0 pg/uL of unlabeled 1,2,3,4-TCDD.

8. SYSTEM PERFORMANCE CRITERIA

System performance criteria are presented below. The laboratory may use any of the recommended columns described in Section 6.2. It must be documented that all applicable system performance criteria specified in Sections 8.1, 8.2, 8.3 and 8.5 have been met before analysis of any sample is performed. Table 2 provides recommended conditions that can be used to satisfy the required criteria. Table 3 provides a typical 12-hour analysis sequence. The GC column performance and mass resolution checks must be performed at the beginning and end of each 12-hour period of operation.

8.1 GC Column Performance

8.1.1 Inject 2 uL (Section 6.1.1) of the column performance check solution (Section 7.7) and acquire selected ion monitoring (SIM) data for m/z 258.930, 319.897, 321.894, 331.937 and 333.934 within a total cycle time of $\leq$1 second (Section 8.3.4.1).

8.1.2 The chromatographic peak separation between 2,3,7,8-TCDD and the peaks representing any other TCDD isomers must be resolved with a valley of $\leq$25 percent, where

$$\text{Valley Percent} = (x/y)(100)$$

x = measured as in Figure 1

y = the peak height of 2,3,7,8-TCDD.

It is the responsibility of the laboratory to verify the conditions suitable for the appropriate resolution of 2,3,7,8-TCDD from all other TCDD isomers. The column performance check solution also contains the TCDD isomers eluting first and last under the analytical conditions specified in this protocol thus defining the retention time window for total TCDD determination. The peaks representing 2,3,7,8-TCDD and the first and the last eluting TCDD isomer must be labeled and identified as such on the chromatograms (F and L, resp.). Any individual selected ion current profile or the reconstructed total ion current (m/z 259 + m/z 320 + m/z 322) constitutes an acceptable form of data presentation.

8.2 Mass Spectrometer Performance

8.2.1 The mass spectrometer must be operated in the electron (impact) ionization mode. Static resolving power of at least 10,000 (10 percent valley) must be demonstrated before any analysis of a set of samples is performed (Section 8.2.2). Static resolution checks must be performed at the beginning and at the end of each 12-hour period of operation. However, it is recommended that a visual check (i.e., not documented) of the static resolution be made using the peak matching unit before and after each analysis.

8.2.2 Chromatography time for TCDD may exceed the long-term mass stability of the mass spectrometer and thus mass drift correction is mandatory. A reference compound [high-boiling perfluorokerosene (PFK) is recommended] is introduced into the mass spectrometer. An acceptable lock mass ion at any mass between m/z 250 and m/z 334 (m/z 318.979 from PFK is recommended) must be used to monitor and correct mass drifts.

NOTE: Excessive PFK may cause background noise problems and contamination of the source resulting in an increase in "downtime" for source cleaning.

Using a PFK molecular leak, tune the instrument to meet the minimum required resolving power of 10,000 (10% valley) at m/z 254.986 (or any other mass reasonably close to m/z 259). Calibrate the voltage sweep at least across the mass range m/z 259 to m/z 334 and verify that m/z 330.979 from PFK (or any other mass close to m/z 334) is measured within ±5 ppm (i.e., 1.7 mmu, if m/z 331 is chosen) using m/z 254.986 as a reference. Documentation of the mass resolution must then be accomplished by recording the peak profile of the PFK reference peak m/z 318.979 (or any other reference peak at a mass close to m/z 320/322). The format of the peak profile representation must allow manual determination of the resolution, i.e., the horizontal axis must be a calibrated mass scale (amu or ppm per division). The result of the peak width measurement (performed at 5 percent of the maximum which corresponds to the 10% valley definition) must appear on the hard copy and cannot exceed 100 ppm (or 31.9 mmu if m/z 319 is the chosen reference ion).

8.3 Initial Calibration

Initial calibration is required before any samples are analyzed for 2,3,7,8-TCDD. Initial calibration is also required if any routine calibration does not meet the required criteria listed in Section 8.6.

8.3.1 All concentration calibration solutions listed in Table 1 must be utilized for the initial calibration.

8.3.2 Tune the instrument with PFK as described in Section 8.2.2.

8.3.3 Inject 2 uL of the column performance check solution (Section 7.7) and acquire SIM mass spectral data for m/z 258.930, 319.897, 321.894, 331.937 and 333.934 using a total cycle time of $\leq$ 1 second (Section 8.3.4.1). The laboratory must not perform any further analysis until it has been demonstrated and documented that the criterion listed in Section 8.1.2 has been met.

8.3.4 Using the same GC (Section 8.1) and MS (Section 8.2) conditions that produced acceptable results with the column performance check solution, analyze a 2-uL aliquot of each of the 4 concentration calibration solutions in triplicate with the following MS operating parameters.

8.3.4.1 Total cycle time for data acquisition must be $\leq$ 1 second. Total cycle time includes the sum of all the dwell times and voltage reset times.

8.3.4.2 Acquire SIM data for the following selected characteristic ions:

m/z	Compound
258.930	TCDD - COCl
319.897	Unlabeled TCDD
321.894	Unlabeled TCDD
331.937	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD
333.934	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD

8.3.4.3 The ratio of integrated ion current for m/z 319.897 to m/z 321.894 for 2,3,7,8-TCDD must be between 0.67 and 0.90.

8.3.4.4 The ratio of integrated ion current for m/z 331.937 to m/z 333.934 for $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD must be between 0.67 and 0.90.

8.3.4.5 Calculate the relative response factors for unlabeled 2,3,7,8-TCDD [RRF(I)] relative to $^{13}C_{12}$-2,3,7,8-TCDD and for labeled $^{13}C_{12}$-2,3,7,8-TCDD [RRF(II)] relative to $^{13}C_{12}$-1,2,3,4-TCDD as follows:

$$RRF(I) = \frac{A_x \cdot Q_{IS}}{Q_x \cdot A_{IS}}$$

$$RRF(II) = \frac{A_{IS} \cdot Q_{RS}}{Q_{IS} \cdot A_{RS}}$$

where

A_x = sum of the integrated ion abundances of m/z 319.897 and m/z 321.894 for unlabeled 2,3,7,8-TCDD.

A_{IS} = sum of the integrated ion abundances of m/z 331.937 and m/z 333.934 for $^{13}C_{12}$-2,3,7,8-TCDD.

A_{RS} = sum of the integrated ion abundances for m/z 331.937 and m/z 333.934 for $^{13}C_{12}$-1,2,3,4-TCDD.

Q_{IS} = quantity of $^{13}C_{12}$-2,3,7,8-TCDD injected (pg).

Q_{RS} = quantity of $^{13}C_{12}$-1,2,3,4-TCDD injected (pg).

Q_x = quantity of unlabeled 2,3,7,8-TCDD injected (pg).

RRF is a dimensionless quantity; the units used to express Q_{IS}, Q_{RS} and Q_x must be the same.

8.3.4.6 Calculate the four means (RRFs) and their respective relative standard deviations (%RSD) for the response factors from each of the triplicate analyses for both unlabeled and $^{13}C_{12}$-2,3,7,8-TCDD (Form H-2).

8.3.4.7 Calculate the grand means $\overline{RRF}(I)$ and $\overline{RRF}(II)$ and their respective relative standard deviations (%RSD) using the four mean RRFs (Section 8.3.4.6) (Form H-2).

8.3.4.8 Calculate the routine calibration permissible range for RRF(I) and RRF(II) using a $\pm$20% window from the grand means $\overline{RRF}(I)$ and $\overline{RRF}(II)$ (Section 8.3.4.7) (Form H-2).

8.4 Criteria for Acceptable Calibration

The criteria listed below for acceptable calibration must be met before analysis of any sample is performed.

8.4.1 The percent relative standard deviation (RSD) for the response factors from each of the triplicate analyses for both unlabeled and $^{13}C_{12}$-2,3,7,8-TCDD must be less than 20 percent.

8.4.2 The variation of the 4 mean RRFs for unlabeled and $^{13}C_{12}$-2,3,7,8-TCDD obtained from the triplicate analyses must be less than 20 percent RSD.

8.4.3 SIM traces for 2,3,7,8-TCDD must present a signal-to-noise ratio of $\geq$2.5 for m/z 258.930, m/z 319.897 and, m/z 321.894.

8.4.4 SIM traces for $^{13}C_{12}$-2,3,7,8-TCDD must present a signal-to-noise ratio $\geq$2.5 for m/z 331.937 and m/z 333.934.

8.4.5 Isotopic ratios (Sections 8.3.4.3 and 8.3.4.4) must be within the allowed range.

NOTE: If the criteria for acceptable calibration listed in Sections 8.4.1 and 8.4.2 have been met, the RRF can be considered independent of the analyte quantity for the calibration concentration range. The mean RRF from 4 triplicate determinations for unlabeled 2,3,7,8-TCDD and for $^{13}C_{12}$-2,3,7,8-TCDD will be used for all calculations until routine calibration criteria (Section 8.6) are no longer met. At such time, new mean RRFs will be calculated from a new set of four triplicate determinations.

8.5 Routine Calibrations

Routine calibrations must be performed at the beginning of a 12-hour period after successful mass resolution and GC column performance check runs.

8.5.1 Inject 2 uL of the concentration calibration solution which contains 10 pg/uL of unlabeled 2,3,7,8-TCDD, 10.0 pg/uL of $^{13}C_{12}$-2,3,7,8-TCDD and 10 pg/uL $^{13}C_{12}$-1,2,3,4-TCDD. Using the same GC/MS/DS conditions as used in Sections 8.1, 8.2 and 8.3, determine and document acceptable calibration as provided in Section 8.6.

8.6 Criteria for Acceptable Routine Calibration

The following criteria must be met before further analysis is performed. If these criteria are not met, corrective action must be taken and the instrument must be recalibrated.

8.6.1 The measured RRF for unlabeled 2,3,7,8-TCDD must be within 20 percent of the mean values established (Section 8.3.4.8) by triplicate analyses of concentration calibration solutions.

8.6.2 The measured RRF for $^{13}C_{12}$-2,3,7,8-TCDD must be within 20 percent of the mean value established by triplicate analysis of the concentration calibration solutions (Section 8.3.4.8).

8.6.3 Isotopic ratios (Sections 8.3.4.3 and 8.3.4.4) must be within the allowed range.

8.6.4 If one of the above criteria is not satisfied, a second attempt can be made before repeating the entire initialization process (Section 8.3).

NOTE: An initial calibration must be carried out whenever the HRCC 2 solution is replaced by a new one from a different lot.

9. QUALITY CONTROL PROCEDURES

See Exhibit E for QA/QC requirements.

10. SAMPLE PRESERVATION AND HANDLING

10.1 Chain-of-custody procedures -- see Exhibit G.

10.2 Sample Preservation

10.2.1 When received, each soil or sediment sample will be contained in a 1-pint glass jar surrounded by vermiculite in a sealed metal paint can. Until a portion is to be removed for analysis, store the sealed paint cans in a locked limited-access area where the temperature is maintained between 25° and 35°C. After a portion of a sample has been removed for analysis, return the remainder of the sample to its original container and store as stated above.

10.2.2 Each aqueous sample will be contained in a 1-liter glass bottle. The bottles with the samples are stored at 4°C in a refrigerator located in a locked limited-access area.

10.2.3 To avoid photodecomposition, protect samples from light.

10.3 Sample Handling

CAUTION: Finely divided soils and sediments contaminated with 2,3,7,8-TCDD are hazardous because of the potential for inhalation or ingestion of particles containing 2,3,7,8-TCDD. Such samples should be handled in a confined environment (i.e., a closed hood or a glove box).

10.3.1 Pre-extraction sample treatment

10.3.1.1 Homogenization -- Although sampling personnel will attempt to collect homogeneous samples, the contractor shall examine each sample and judge if it needs further mixing.

NOTE: Contractor personnel have the responsibility to take a representative sample portion; this responsibility entails efforts to make the sample as homogeneous as possible. Stirring is recommended when possible.

10.3.1.2 Centrifugation -- When a soil or sediment sample contains an obvious liquid phase, it must be centrifuged to separate the liquid from the solid phase. Place the entire sample in a suitable centrifuge bottle and centrifuge for 10 minutes at 2000 rpm. Remove the bottle from the centrifuge. With a disposable pipet, remove the liquid phase and discard it. Mix the solid phase with a stainless steel spatula and remove a portion to be weighed and analyzed. Return the remaining solid portion to the original sample bottle (which must be empty) or to a clean, empty sample bottle which is properly labeled, and store it as described in 10.2.1.

CAUTION: The removed liquid may contain TCDD and should be disposed as a liquid waste.

10.3.1.3 Weigh between 9.5 and 10.5 g of the soil or sediment sample (±0.5 g) to 3 significant figures. Dry it to constant weight at 100°C. Allow the sample to cool in a desiccator. Weigh the dried soil to 3 significant figures. Calculate and report percent moisture on Form H-9.

11. SAMPLE EXTRACTION

11.1 Soil/Sediment Extraction

11.1.1 Immediately before use, the Soxhlet apparatus is charged with 200 to 250 mL benzene which is then refluxed for 2 hours. The apparatus is allowed to cool, disassembled and the benzene removed and retained as a blank for later analysis if required.

11.1.2 Accurately weigh to 3 significant figures a 10-g (9.50 g to 10.50 g) portion of the wet soil or sediment sample. Mix 100 uL of the sample fortification solution (Section 7.8) with 1.5 mL acetone (1000 pg of $^{13}C_{12}$-2,3,7,8-TCDD) and deposit the entire mixture in small portions on several sites on the surface of the soil or sediment.

11.1.3 Add 10 g anhydrous sodium sulfate and mix thoroughly using a stainless steel spoon spatula.

11.1.4 After breaking up any lumps, place the soil-sodium sulfate mixture in the Soxhlet apparatus using a glass wool plug (the use of an extraction thimble is optional). Add 200 to 250 mL benzene to the Soxhlet apparatus and reflux for 24 hours. The solvent must cycle completely through the system at least 3 times per hour.

11.1.5 Transfer the extract to a Kuderna-Danish apparatus and concentrate to 2 to 3 mL. Rinse the column and flask with 5 mL benzene and collect the rinsate in the concentrator tube. Reduce the volume in the concentrator tube to 2 to 3 mL. Repeat this rinsing and concentrating operation twice more. Remove the concentrator tube from the K-D apparatus and carefully reduce the extract volume to approximately 1 mL with a stream of nitrogen using a flow rate and distance such that gentle solution surface rippling is observed.

NOTE: Glassware used for more than one sample must be carefully cleaned between uses to prevent cross-contamination (Note on page D-6).

11.2 Extraction of Aqueous Samples

11.2.1 Mark the water meniscus on the side of the 1-L sample bottle for later determination of the exact sample volume. Pour the entire sample (approximately 1 L) into a 2-L separatory funnel.

11.2.2 Mix 100 uL of the sample fortification solution with 1.5 mL acetone (1000 pg of $^{13}C_{12}$-2,3,7,8-TCDD) and add the mixture to the sample in the separatory funnel.

NOTE: A continuous liquid-liquid extractor may be used in place of a separatory funnel.

11.2.3 Add 60 mL methylene chloride to the sample bottle, seal and shake 30 seconds to rinse the inner surface. Transfer the solvent to the separatory funnel and extract the sample by shaking the funnel for 2 minutes with periodic venting. Allow the organic layer to separate from the water phase for a minimum of 10 minutes. If an emulsion interface between layers exists, the analyst must employ mechanical techniques (to be described in the final report) to complete the phase separation. Collect the methylene chloride (3 x 60 mL) directly into a 500-mL Kuderna-Danish concentrator (mounted with a 10-mL concentrator tube) by passing the sample extracts through a filter funnel packed with a glass wool plug and 5 g of anhydrous sodium sulfate. After the third extraction, rinse the sodium sulfate with an additional 30 mL of methylene chloride to ensure quantitative transfer.

11.2.4 Attach a Snyder column and concentrate the extract until the apparent volume of the liquid reaches 1 mL. Remove the K-D apparatus and allow it to drain and cool for at least 10 minutes. Remove the Snyder column, add 50 mL benzene, reattach the Snyder column and concentrate to approximately 1 mL. Rinse the flask and the lower joint with 1 to 2 mL benzene. Concentrate the extract to 1.0 mL under a gentle stream of nitrogen.

11.2.5 Determine the original sample volume by refilling the sample bottle to the mark and transferring the liquid to a 1000-mL graduated cylinder. Record the sample volume to the nearest 5 mL.

11.3 Cleanup Procedures

11.3.1 Prepare an acidic silica column as follows: Pack a 1 cm x 10 cm chromatographic column with a glass wool plug, a layer (1 cm) of Na_2SO_4/K_2CO_3(1:1), 1.0 g silica gel (Section 7.1.2) and 4.0 g of 40-percent w/w sulfuric acid-impregnated silica gel (Section 7.1.3). Pack a second chromatographic column (1 cm x 30 cm) with a glass wool plug, 6.0 g acidic alumina (Section 7.1.1) and top with a 1-cm layer of sodium sulfate (Section 7.4.1). Add hexane to the columns until they are free of channels and air bubbles.

11.3.2 Quantitatively transfer the benzene extract (1 mL) from the concentrator tube to the top of the silica gel column. Rinse the concentrator tube with two 0.5-mL portions of hexane. Transfer the rinses to the top of the silica gel column.

11.3.3 Elute the extract from the silica gel column with 90 mL hexane directly into a Kuderna-Danish concentrator. Concentrate the eluate to 0.5 mL, using nitrogen blow-down as necessary.

11.3.4 Transfer the concentrate (0.5 mL) to the top of the alumina column. Rinse the K-D assembly with two 0.5-mL portions of hexane and transfer the rinses to the top of the alumina column. Elute the alumina column with 18 mL hexane until the hexane level is just below the top of the sodium sulfate. Discard the eluate. Columns must not be allowed to reach dryness (i.e., a solvent "head" must be maintained.)

11.3.5 Place 30 mL of 20-percent (v/v) methylene chloride in hexane on top of the alumina and elute the TCDDs from the column. Collect this fraction in a 50-mL Erlenmeyer flask.

11.3.6 Prepare an 18-percent Carbopak C/Celite 545® mixture by thoroughly mixing 3.6 grams Carbopak C (80/100 mesh) and 16.4 grams Celite 545® in a 40-mL vial. Activate at 130°C for 6 hours. Store in a desiccator. Cut off a clean 5-mL disposable glass pipet (6 to 7mm ID) at the 4-mL mark. Insert a plug of glass wool (Section 7.3) and push to the 2-mL mark. Add 340 to 600 mg of the activated Carbopak/Celite mixture (see NOTE) followed by another glass wool plug. Using two glass rods, push both glass wool plugs simultaneously towards the Carbopak/Celite mixture and gently compress the Carbopak/Celite plug to a length of 2 to 2.5 cm. Preelute the column with 2 mL toluene followed by 1 mL of 75:20:5 methylene chloride/methanol/benzene, 1 mL of 1:1 cyclohexane in methylene chloride, and 2 mL hexane. The flow rate should be less than 0.5 mL/min. While the column is still wet with hexane, add the entire eluate (30 mL) from the alumina column (Section 11.3.5) to the top of the column. Rinse the Erlenmeyer flask which contained the extract twice with 1 mL hexane and add the rinsates to the top of the column. Elute the column sequentially with two 1-mL aliquots hexane, 1 mL of 1:1 cyclohexane in methylene chloride, and 1 mL of 75:20:5 methylene chloride/ methanol/benzene. Turn the column upside down and elute the TCDD fraction with 6 mL toluene into a concentrator tube. Warm the tube to approximately 60°C and reduce the toluene volume to approximately 1 mL using a stream of nitrogen. Carefully transfer the concentrate into a 1-mL mini-vial and, again at elevated temperature, reduce the volume to about 100 uL using a stream of nitrogen. Rinse the concentrator tube with 3 washings using 200 uL of 1% toluene in CH_2Cl_2. Add 10 uL of the tridecane solution containing the recovery standard and store the sample in a refrigerator until HRGC/HRMS analysis is performed.

NOTE: The amount of activate Carbopak/Celite mixture required to form a 2-to 2.5-cm plug in the column depends on the density of the Celite being used.

12. ANALYTICAL PROCEDURES

12.1 Remove the sample extract or blank from storage and allow it to warm to ambient laboratory temperature. With a stream of dry, purified nitrogen, reduce the extract/blank volume to 10 uL.

12.2 Inject a 2-uL aliquot of the extract into the GC, operated under the conditions previously used (Section 8.1) to produce acceptable results with the performance check solution.

12.3 Acquire SIM data according to 12.3.1. Use the same acquisition and MS operating conditions previously used (Section 8.3.4) to determine the relative response factors.

12.3.1 Acquire SIM data for the following selected characteristic ions:

m/z	Compound
258.930	TCDD - COCl
319.897	Unlabeled TCDD
321.894	Unlabeled TCDD
331.937	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD
333.934	$^{13}C_{12}$-2,3,7,8-TCDD, $^{13}C_{12}$-1,2,3,4-TCDD

NOTE: The acquisition period must at least encompass the TCDD retention time window previously determined (Section 8.1.2, Exhibit D).

12.4 Identification Criteria

12.4.1 The retention time (RT) (at maximum peak height) of the sample component m/z 319.897 must be within -1 to +3 seconds of the retention time of the peak for the isotopically labeled internal standard at m/z 331.937 to attain a positive identification of 2,3,7,8-TCDD. Retention times of other tentatively identified TCDDs must fall within the RT window established by analyzing the column performance check solution (Section 8.1). Retention times are required for all chromatograms.

12.4.2 The ion current responses for m/z 258.930, 319.897 and 321.894 must reach maximum simultaneously ($\pm$ 1 sec), and all ion current intensities must be $\geq$ 2.5 times noise level for positive identification of a TCDD or group of coeluting TCDD isomers.

12.4.3 The integrated ion current at m/z 319.897 must be between 67 and 90 percent of the ion current response at m/z 321.894.

12.4.4 The integrated ion current at m/z 331.937 must be between 67 and 90 percent of the ion current response at m/z 333.934.

12.4.5 The integrated ion currents for m/z 331.937 and 333.934 must reach their maxima within $\pm$ 1 sec.

12.4.6 The recovery of the internal standard ${}^{13}C_{12}$-2,3,7,8-TCDD must be between 40 and 120 percent.

13. CALCULATIONS

13.1 Calculate the concentration of 2,3,7,8-TCDD (or any other TCDD isomer or group of coeluting TCDD isomers) using the formula:

$$C_X = \frac{A_X \cdot Q_{IS}}{A_{IS} \cdot W \cdot \overline{RRF}(I)}$$

where:

C_X = unlabeled 2,3,7,8-TCDD (or any other unlabeled TCDD isomer or group of coeluting TCDD isomers) concentration in pg/g.

A_X = sum of the integrated ion abundances determined for m/z 319.897 and 321.894.

A_{IS} = sum of the integrated ion abundances determined for m/z 331.937 and 333.934 of ${}^{13}C_{12}$-2,3,7,8-TCDD (IS = internal standard).

Q_{IS} = quantity (in picograms) of $^{13}C_{12}$-2,3,7,8-TCDD added to the sample before extraction (Q_{IS} = 1000 pg).

W = weight (in grams) of dry soil or sediment sample or volume of aqueous sample converted to grams.

$\overline{RRF}(I)$ = calculated mean relative response factor for unlabeled 2,3,7,8-TCDD relative to $^{13}C_{12}$-2,3,7,8-TCDD. This represents the grand mean of the RRF(I)'s obtained in Section 8.3.4.5.

13.2 Calculate the recovery of the internal standard $^{13}C_{12}$-2,3,7,8-TCDD measured in the sample extract, using the formula:

$$\text{Internal standard percent recovery} = \gamma \frac{A_{IS}}{A_{RS} \cdot \overline{RRF}(II)} \cdot 100$$

Where:

A_{IS} = sum of the integrated ion abundances determined for m/z 331.937 and 333.934 of $^{13}C_{12}$-2,3,7,8-TCDD (IS = internal standard).

A_{RS} = sum of the integrated ion abundances determined for m/z 331.937 and 333.934 of $^{13}C_{12}$-1,2,3,4-TCDD (RS = recovery standard).

γ = 0.1 for the "10-µL extract" injection (to be reported on Forms H-1, H-5 and H-9).

and γ = 1.2 for the "24-µL extract" injection (Section 13.3) (to be reported on Form H-9 used for reporting the diluted extract analysis).

$\overline{RRF}(II)$ = calculated mean relative response factor for labeled $^{13}C_{12}$-2,3,7,8-TCDD relative to $^{13}C_{12}$-1,2,3,4-TCDD. This represents the grand mean of the RRF(II)'s calculated in Section 8.3.4.5.

13.3 If the concentration of the most abundant TCDD isomer (or group of coeluting TCDD isomers) exceeds 100 pg/uL in the 10 uL final extract, the linear range of response vs. concentration may have been exceeded, and a diluted aliquot of the original sample extract must be analyzed. Accurately dilute 2 uL of the remaining original extract with 22 uL of the tridecane solution containing 10 pg/uL of the recovery standard (Section 7.9, Exhibit D).

13.4 Total TCDD concentration -- all positively identified isomers of TCDD must be within the RT window and meet all identification criteria listed in Sections 12.4.2 and 12.4.3. Use the expression in Section 13.1 to calculate the concentrations of the other TCDD isomers, with C_x becoming the concentration of any unlabeled TCDD isomer or group of coeluting TCDD isomers.

C Total TCDD = Sum of the concentrations of the individual TCDDs including 2,3,7,8-TCDD.

13.5 Estimated Detection Limit -- For samples in which no unlabeled 2,3,7,8-TCDD was detected, calculate the estimated minimum detectable concentration. The background area is determined by integrating the ion abundances for m/z 319.897 and 321.894 in the appropriate region of the selected ion current profiles, multiplying that area by 2.5, and relating the product area to an estimated concentration that would produce that product area.

Use the formula:

$$C_E = \frac{(2.5) \cdot (A_x) \cdot (Q_{IS})}{(A_{IS}) \cdot (\overline{RRF}(I)) \cdot (W)}$$

where

C_E = estimated concentration of unlabeled 2,3,7,8-TCDD required to produce A_x.

A_x = sum of integrated ion abundances for m/z 319.897 and 321.894 in the same group of ≥ 5 scans used to measure A_{IS}.

A_{IS} = sum of integrated ion abundances for the appropriate ion characteristic of the internal standard, m/z 331.937 and m/z 333.934.

Q_{IS}, $\overline{RRF}(I)$, and W retain the definitions previously stated in Section 13.1. Alternatively, if peak height measurements are used for quantification, measure the estimated detection limit by the peak height of the noise in the 2,3,7,8-TCDD RT window.

13.6 The relative percent difference (RPD) is calculated as follows:

$$\text{RPD} = \frac{|S_1 - S_2|}{\text{Mean Concentration}} = \frac{|S_1 - S_2|}{(S_1 + S_2)/2} \times 100$$

S_1 and S_2 represent sample and duplicate sample results.

References

1. "Carcinogens - Working with Carcinogens", Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, National Institute for Occupational Safety and Health, Publication No. 77-206, Aug. 1977.

2. "OSHA Safety and Health Standards, General Industry" (29 CFR1910), Occupational Safety and Health Administration, OSHA 2206 (Revised January 1976).

3. "Safety in Academic Chemistry Laboratories", American Chemical Society Publication, Committee on Chemical Safety, 3rd Edition 1979.

TABLE 1. COMPOSITION OF CONCENTRATION CALIBRATION SOLUTIONS

	Recovery Standard $^{13}C_{12}$-1,2,3,4-TCDD	Analyte 2,3,7,8-TCDD	Internal Standard $^{13}C_{12}$-2,3,7,8-TCDD
HRCC1	2.0 pg/uL	2.0 pg/uL	10.0 pg/uL
HRCC2	10.0 pg/uL	10.0 pg/uL	10.0 pg/uL
HRCC3	50.0 pg/uL	50.0 pg/uL	10.0 pg/uL
HRCC4	100.0 pg/uL	100.0 pg/uL	10.0 pg/uL

Sample Fortification Solution

10.0 pg/uL of $^{13}C_{12}$-2,3,7,8-TCDD

Recovery Standard Spiking Solution

10.0 pg/uL $^{13}C_{12}$-1,2,3,4-TCDD

Field Blank Fortification Solutions

A) 10.0 pg/uL of unlabeled 2,3,7,8-TCDD

B) 10.0 pg/uL of unlabeled 1,2,3,4-TCDD

Internal Standard Spiking Solution

10 pg/uL of $^{13}C_{12}$-2,3,7,8-TCDD
(Used only in Section 4.2.1.1, Exhibit E)

TABLE 2. RECOMMENDED GC OPERATING CONDITIONS

Column coating	SP-2330 (SP-2331)	CP-SIL 88
Film thickness	0.2 um	0.22 um
Column dimensions	60 m x 0.24 mm	50 m x 0.22 mm
Helium linear velocity	28-29 cm/sec at 240°C	28-29 cm/sec at 240°C
Initial temperature	150°C	200°C
Initial time	4 min	1 min
Temperature program	Rapid increase to 200°C (15°C/min) 200°C to 250°C at 4°C/min	Program from 200°C to 240°C at 4°C/min
Approximate 2,3,7,8-TCDD retention time	27 min	22 min

TABLE 3. TYPICAL 12-HOUR SEQUENCE FOR 2,3,7,8-TCDD ANALYSIS

1.	Static mass resolution check and mass measurement error determination	10/20/84	0700h
2.	Column performance check	10/20/84	0730h
3.	HRCC2	10/20/84	0800h
4.	Sample 1 through Sample "N"	10/20/84	0830h
5.	Column performance check	10/20/84	1800h
6.	Static mass resolution check	10/20/84	1830h

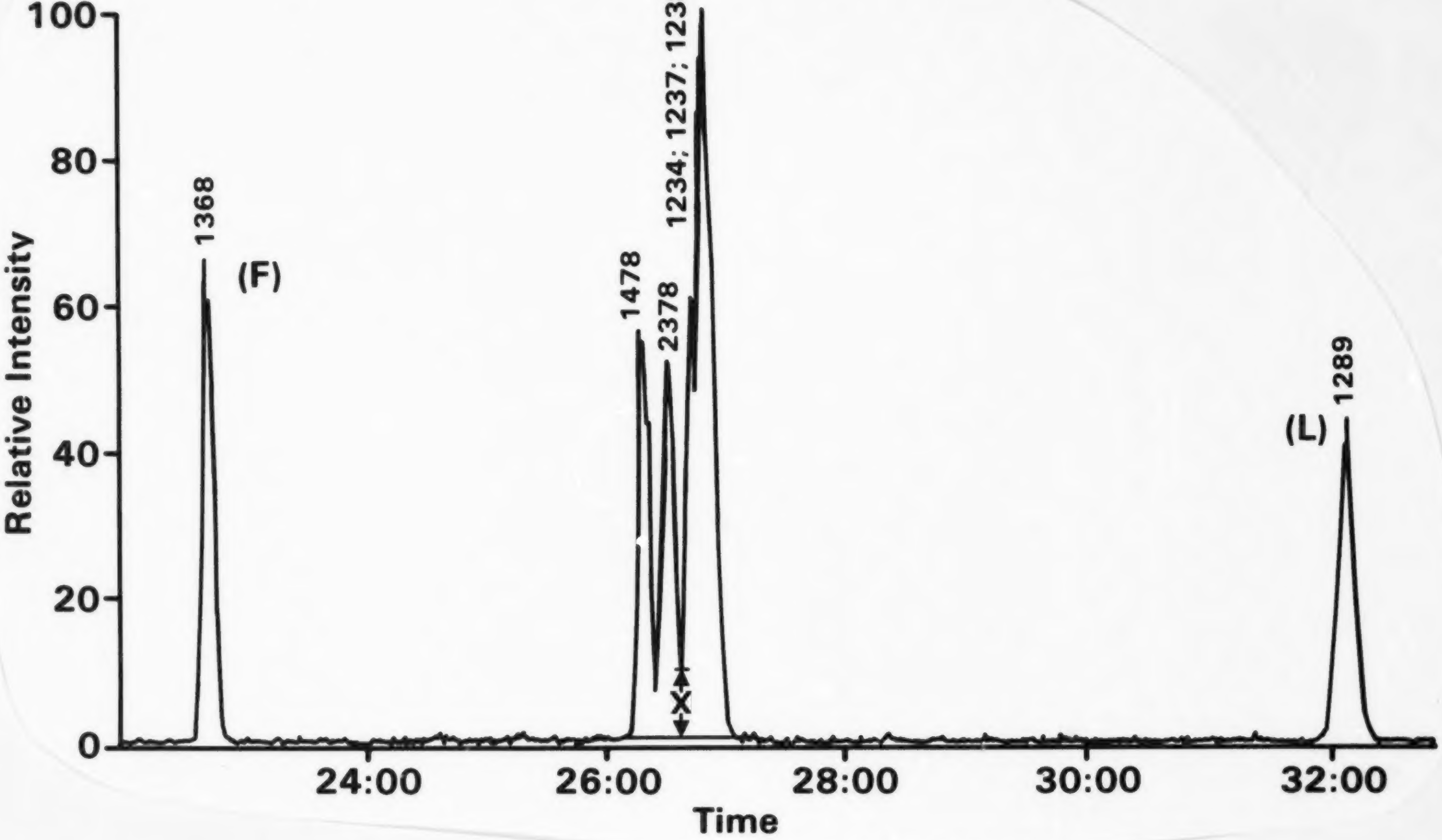

Figure 1. Selected ion current profile for m/z 322 produced by MS analysis of performance check solution using a 60-m SP-2331 fused silica capillary column and conditions listed in Table 2.

EXHIBIT E

QA/QC Requirements

SUMMARY OF QC ANALYSES

- Initial and periodic calibration and instrument performance checks.
- Field blank analyses (Section 4.1); a minimum of one fortified field blank pair shall be analyzed with each sample batch; an additional fortified field blank pair must be analyzed when a new lot of absorbent and/or solvent is used.
- Analysis of a batch of samples with accompanying QC analyses:

  Sample Batch -- ≤24 samples, including field blank and rinsate sample(s).

  Additional QC analyses per batch:

Fortified field blanks	2
Method blank	(1*)
Duplicate sample	1
TOTAL	3(4)

* A method blank is required whenever a fortified field blank shows a positive response as defined in Section 3.11, Exhibit D.

- "Blind" QC samples may be submitted to the contractor as ordinary soil, sediment or water samples included among the batch of samples. Blind samples include:

  Uncontaminated soil, sediment and water,

  Split samples,

  Unidentified duplicates, and

  Performance evaluation samples.

QUALITY CONTROL

1. Performance Evaluation Samples -- Included among the samples in all batches will be samples containing known amounts of unlabeled 2,3,7,8-TCDD and/or other TCDDs that may or may not be marked as other-than-ordinary samples.

2. Performance Check Solutions

   2.1 At the beginning of each 12-hour period during which samples are to be analyzed, an aliquot each of the 1) GC column performance check solution and 2) high-resolution concentration calibration solution

No. 2 (HRCC2) shall be analyzed to demonstrate adequate GC resolution and sensitivity, response factor reproducibility, and mass range calibration. A mass resolution check shall also be performed to demonstrate adequate mass resolution using an appropriate reference compound (PFK is recommended).

These procedures are described in Section 8 of Exhibit D. If the required criteria are not met, remedial action must be taken before any samples are analyzed.

2.2 To validate positive sample data, the GC column performance check and the mass resolution check must be performed also at the end of each 12-hour period during which samples are analyzed.

2.2.1 If the contractor laboratory operates only during one period (shift) each day of 12 hours or less, the GC performance check solution must be analyzed twice (at the beginning and end of the period) to validate data acquired during the interim period. This applies also to the mass resolution check.

2.2.2 If the contractor laboratory operates during consecutive 12-hour periods (shifts), analysis of the GC performance check solution at the beginning of each 12-hour period and at the end of the final 12-hour period is sufficient. This applies also to the mass resolution check.

2.3 Results of at least two analyses of the GC column performance check solution and the mass resolution check must be reported with the sample data collected during a 12-hour period.

2.4 Deviations from criteria specified for the GC performance check or for the mass resolution check (Section 8, Exhibit D) invalidate all positive sample data collected between analyses of the performance check solution, and the extract from those positive samples shall be reanalyzed Exhibit C).

3. The GC column performance check mixture, concentration calibration solutions, and the sample fortification solutions are to be obtained from the EMSL-LV. However, if not available from the EMSL-LV, standards can be obtained from other sources, and solutions can be prepared in the contractor laboratory. Concentrations of all solutions containing unlabeled 2,3,7,8-TCDD which are not obtained from the EMSL-LV must be verified by comparison with the unlabeled 2,3,7,8-TCDD standard solution (concentration of 7.87 ug/mL) that is available from the EMSL-LV. When a lower-concentration standard solution becomes available from the EMSL-LV, it will be substituted for the 7.87 ug/mL standard.

4. Blanks

4.1 A method blank is required whenever a positive response (Section 3.11, Exhibit D) is obtained for a fortified field blank. To that effect, perform all steps detailed in the analytical procedure (Section 11, Exhibit D) using all reagents, standards, equipment, apparatus, glassware, and solvents that would be used for a sample analysis, but omit addition of the soil, sediment or aqueous sample portion.

4.1.1 The method blank must contain the same amount of ${}^{13}C_{12}$-2,3,7,8-TCDD that is added to samples before extraction.

4.1.2 An acceptable method blank exhibits no positive response (Section 3.11, Exhibit D) for any of the characteristic ions monitored. If the method blank which was extracted along with a batch of samples is contaminated, all positive samples must be rerun (Exhibit C).

4.1.2.1 If the above criterion is not met, check solvents, reagents, fortification solutions, apparatus, and glassware to locate and eliminate the source of contamination before any samples are extracted and analyzed.

4.1.2.2 If new batches of reagents or solvents contain interfering contaminants, purify or discard them.

4.2 Field blanks -- Each batch of samples contains a field blank sample of uncontaminated soil/sediment or water that is to be fortified before analysis according to Section 4.2.1, Exhibit E. In addition to this field blank, a batch of samples may include a rinsate, that is a portion of solvent (usually trichloroethylene) that was used to rinse sampling equipment. The rinsate is analyzed to assure that the samples have not been contaminated by the sampling equipment.

4.2.1 Fortified field blank pair

4.2.1.1 Fortified field blank A: 2,3,7,8-TCDD

4.2.1.1.1 Weigh a 10-g portion or use 1 liter (for aqueous samples) of the specified field blank sample and add 100 uL of the solution containing 10.0 pg/uL of 2,3,7,8-TCDD (Table 1, Exhibit D) diluted in 1.5 mL of acetone (Section 11.1.2, Exhibit D).

4.2.1.1.2 Extract using the procedures beginning in Sections 11.1 or 11.2 of Exhibit D, as applicable, add 10 uL of the internal standard solution (Section 7.10, Exhibit D) and analyze a 2-uL aliquot of the concentrated extract.

NOTE: This is the only case where the recovery standard is used for other than recovery purposes.

4.2.1.1.3 Calculate the concentration (Section 13.1, Exhibit D) of 2,3,7,8-TCDD and the percent recovery of unlabeled 2,3,7,8-TCDD. If the percent recovery at the measured concentration of 2,3,7,8-TCDD is <40 percent or >120 percent, report the results and repeat the fortified field blank extraction and analysis with a second aliquot of the specified field blank sample (Exhibit C).

4.2.1.1.4 Extract and analyze a new fortified simulated field blank whenever new lots of solvents or reagents are used for sample extraction or for column chromatographic procedures. When a fortified simulated field blank produces a positive response (Section 3.11, Exhibit D) for any m/z being monitored at the retention time of 1,2,3,4-TCDD, a method blank (Section 4.1, Exhibit E) is required.

NOTE: For this purpose only, the Contractor will simulate field blanks by using clean sand or distilled water.

4.2.1.2 Fortified field blank B: 1,2,3,4-TCDD

4.2.1.2.1 Repeat steps 4.2.1.1.1 to 4.2.1.1.3 using unlabeled 1,2,3,4TCDD (instead of 2,3,7,8-TCDD) and $^{13}C_{12}$-1,2,3,4-TCDD (instead of $^{13}C_{12}$-2,3,7,8-TCDD) as recovery standard.

4.2.1.2.2 Extract and analyze a new fortified simulated field blank whenever new lots of solvents or reagents are used for sample extraction or for column chromatographic procedures. When a fortified simulated field blank produces a positive response (Section 3.11, Exhibit D) for any m/z being monitored at the retention time of 2,3,7,8-TCDD, a method blank (Section 4.1, Exhibit E) is required.

4.2.2 Rinsate sample

4.2.2.1 The rinsate sample must be fortified as a regular sample.

4.2.2.2 Take a 100-mL aliquot of sampling equipment rinse solvent (rinsate sample), filter, if necessary, and add 100 uL of the solution containing 10.0 pg/uL of $^{13}C_{12}$-2,3,7,8-TCDD (Table 1, Exhibit D).

4.2.2.3 Using a Kuderna-Danish apparatus, concentrate to approximately 5 mL.

4.2.2.4 Transfer the 5-mL concentrate in 1-mL portions to a 1-mL mini-vial, reducing the volume as necessary with a gentle stream of dry nitrogen; see Exhibit D, Section 11.1.5 for volume reduction procedures.

4.2.2.5 Rinse the container with two 0.5-mL portions of hexane and transfer the rinses to the 1-mL mini-vial.

4.2.2.6 Just before analysis, add 10 uL tridecane recovery standard spiking solution (Table 1, Exhibit D), and reduce the volume to a final volume of 10 uL (no column chromatography is required).

4.2.2.7 Analyze an aliquot following the same procedures used to analyze samples (Section 12, Exhibit D).

4.2.2.8 Report percent recovery of the internal standard and the level of contamination by any TCDD isomer (or group of coeluting TCDD isomers) on Form H-5 in pg/mL of rinsate solvent.

5. Duplicate Analyses

5.1 Laboratory duplicates -- in each batch of samples, locate the sample specified for duplicate analysis and analyze a second 10-g soil or sediment sample portion or 1-L water sample.

5.1.1 The results of laboratory duplicates (percent recovery and concentrations of 2,3,7,8-TCDD and total TCDD) must agree within 50 percent relative difference (difference expressed as percentage of the mean). If the relative difference is >50 percent, the Contractor shall immediately contact the Sample Management Office for resolution of the problem. Report all results.

5.1.2 Recommended actions to help locate problems:

5.1.2.1 Verify satisfactory instrument performance (Section 8, Exhibit D).

5.1.2.2 If possible, verify that no error was made while weighing sample portions.

5.1.2.3 Review the analytical procedures with the performing laboratory personnel.

6. Percent Recovery of the Internal Standard $^{13}C_{12}$-2,3,7,8-TCDD -- For each sample, method blank and rinsate, calculate the percent recovery (Section 13.2, Exhibit D) of the measured concentration of $^{13}C_{12}$-2,3,7,8-TCDD. If

the percent recovery is <40 percent or >120 percent for a sample, analyze a second portion of that sample and report both results (Exhibit C).

NOTE: A low or high percent recovery for a blank does not require discarding analytical data but it may indicate a potential problem with future analytical data.

7. Identification Criteria

7.1 If either of the two identification criteria (Sections 12.4.1 and 12.4.2, Exhibit D) is not met, it is reported that the sample does not contain unlabeled 2,3,7,8-TCDD at the calculated detection limit (Section 13.5, Exhibit D).

7.2 If the first two initial identification criteria are met, but the third, fourth, fifth or sixth criterion (Sections 12.4.3 through 12.4.6, Exhibit D) is not met, that sample is presumed to contain interfering contaminants. This must be noted on the analytical report form and the sample must be rerun or the extract reanalyzed. Detailed sample rerun and extract reanalysis requirements are presented in Exhibit C.

8. Blind QC Samples -- Included among soil, sediment and aqueous samples may be QC samples that are not specified as such to the performing laboratory. Types that may be included are:

8.1 Uncontaminated soil, sediment or water.

8.1.1 If a false positive is reported for such a sample, the Contractor shall be required to rerun the entire associated batch of samples (Section 2.3.3, Exhibit C).

8.2 Split samples -- composited sample portions sent to more than one laboratory.

8.3 Unlabeled field duplicates -- two portions of a composited sample.

8.4 Performance evaluation sample -- soil/sediment or water sample containing a known amount of unlabeled 2,3,7,8-TCDD and/or other TCDDs.

8.4.1 If the performance evaluation sample result falls outside the acceptance windows established by EPA, the Contractor shall be required to rerun the entire associated batch of samples (Exhibit C).

NOTE: EPA acceptance windows are based on previously generated data.

9. Records - At each contractor laboratory, records must be maintained on

site for six months after contract completion to document the quality of all data generated during the contract performance. Before any records are disposed, written concurrence from the Contracting Officer must be obtained.

10. Unused portions of samples and sample extracts must be preserved for six months after sample receipt; appropriate samples may be selected by EPA personnel for further analyses.

11. Reuse of glassware is to be minimized to avoid the risk of contamination.

LABORATORY EVALUATION PROCEDURES

1. On a quarterly basis, the EPA Project Officer and/or designated representatives may conduct an evaluation of the laboratory to ascertain that the laboratory is meeting contract requirements. This section outlines the procedures which may be used by the Project Officer or his authorized representative in order to conduct a successful evaluation of laboratories conducting dioxin analyses according to this protocol. The evaluation process consists of the following steps: 1) analysis of a performance evaluation (PE) sample, and 2) on-site evaluation of the laboratory to verify continuity of personnel, instrumentation, and quality assurance/quality control functions. The following is a description of these two steps.

2. Performance Evaluation Sample Analysis

   2.1 The PE sample set will be sent to a participating laboratory to verify the laboratory's continuing ability to produce acceptable analytical results. The PE sample will be representative of the types of samples that will be subject to analysis under this contract.

   2.2 When the PE sample results are received, they are scored using the PE Sample Score Sheet shown in Figure 1. If a false positive (e.g., a PE sample not containing 2,3,7,8-TCDD and/or other TCDDs but reported by the laboratory to contain it and/or them) is reported, the laboratory has failed the PE analysis requirement. The Project Officer will notify the laboratory immediately if such an event occurs.

   2.3 As a general rule, a laboratory should achieve 75 percent or more of the total possible points for all three categories, and 75 percent or more of the maximum possible points in each category to be considered acceptable for this program. However, the Government reserves the right to accept scores of less than 75 percent.

   2.4 If unanticipated difficulties with the PE samples are encountered, the total points may be adjusted by the Government evaluator in an impartial and equitable manner for all participating laboratories.

Number of PE Samples	Maximum Possible Score	Recommended Passing Score (75%)
1	290	218
2	475	356
3	660	495
4	845	634
5	1030	773

3. On-Site Laboratory Evaluation

3.1 An on-site laboratory evaluation is performed to verify that (1) the laboratory is maintaining the necessary minimum level in instrumentation and levels of experience in personnel committed to the contract and (2) that the necessary quality control/quality assurance activities are being carried out. It also serves as a mechanism for discussing laboratory weaknesses identified through routine data audits, PE sample analyses results, and prior on-site evaluation. Photographs may be taken during the on-site laboratory evaluation tour.

3.2 The sequence of events for the on-site evaluations is shown in Figure 2. The Site Evaluation Sheet (SES) (Figure 3) is used to document the results of the evaluation.

PERFORMANCE EVALUATION SAMPLE SCORE SHEET

Laboratory ______________________________ Date ______________

	False Positive
I. False Positive - If a laboratory reports a false positive on any PE sample, the laboratory may be disqualified, i.e., rendered ineligible for contract award based on the failure to pass the PE sample analysis requirement.	
2,3,7,8-TCDD	( ) Yes ( ) No
Other TCDD(s)	( ) Yes ( ) No

	Possible Score	Score Achieved
II. Calibration Data		
1. Method Blank:		
a. Results properly recorded on Forms H-1, H-5 and H-9.	5	
b. No native TCDD isomers at/or above method quantitative limit.	5	
c. Results documented by selected ion monitoring (SIM) traces for m/z being monitored to detect TCDDs.	5	
d. Percent recovery of $^{13}C_{12}$-2,3,7,8-TCDD $\geq$40 and $\leq$120%.	5	
2. Initial Concentration Calibration:		
a. Results properly recorded on Forms H-2 and H-8.	5	
b. The percent relative standard deviation (RSD) for the response factors for each of the triplicate analyses for both unlabeled and $^{13}C_{12}$-2,3,7,8-TCDD less than 20%.	5	
c. The variation of the 4 mean RRFs for both unlabeled and labeled 2,3,7,8-TCDD obtained from the triplicate analyses less than 20% RSD.	5	
d. For unlabeled 2,3,7,8-TCDD the abundance ratio must be $\geq$0.67 and $\leq$0.90 for m/z 319.897 to 321.894.	5	

Figure 1. Performance evaluation sample score sheet.

	Possible Score	Score Achieved
e. The abundance ratios must be $\geq$0.67 and $\leq$0.90 for 331.937 to 333.934 for $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD.	5	
f. Results must be documented with appropriate SIM traces, labeled with the corresponding EPA sample numbers, and calculations.	5	
3. Performance Checks:		
a. GC resolution and MS resolution checks performed at the beginning and end of each 12-hour period.	5	
b. Results of performance checks properly recorded on Form H-4.	5	
c. MS Resolution: PFK (or alternate) tune shows appropriate mass resolution (Section 8.2, Exhibit D) with mass assignment accuracy within ±5 ppm.	5	
d. GC Resolution: chromatograms meet the criteria specified in Section 8.1, Exhibit D.	5	
4. Routine Calibration:		
a. Performed each 12 hours, after MS and GC resolution checks, using HRCC2.	5	
b. Results of routine calibrations properly reported on Forms H-3 and H-8.	5	
c. For unlabeled 2,3,7,8-TCDD: abundance ratio must be $\geq$0.67 and $\leq$0.90 for m/z 319.897 to 321.894.	5	
d. Abundance ratio correct for isotopically labeled standards (e.g., 331.937/333.934 must be $\geq$0.67 and $\leq$0.90 for $^{13}C_{12}$-2,3,7,8-TCDD and $^{13}C_{12}$-1,2,3,4-TCDD).	5	
e. Response factors [RRF(I) and RRF(II)] are within ±20% of the mean of the respective initial calibration response factors.	5	
f. Signal-to-Noise (S/N) Ratio: SIM traces for 2,3,7,8-TCDD demonstrate S/N of $\geq$2.5.	5	
g. Results documented with appropriate SIM traces and calculations.	5	
Subtotal II	105	

Figure 1. (Continued).

	Possible Score	Score Achieved
III. Performance Evaluation (PE) Sample Data (Scores to be determined for each sample in the PE set)		
1. Forms H-1 and H-9 properly filled out for sample.	5	
2. Measured concentration of unlabeled 2,3,7,8-TCDD within acceptance window established by EPA.	40	
3. Estimated concentration of total TCDDs within acceptance window established by EPA.	20	
4. Identification Criteria for 2,3,7,8-TCDD:		
a. Retention time (RT) (at maximum peak height) of the sample component m/z 319.897 is within -1 to +3 seconds of the m/z 331.937 $^{13}C_{12}$2,3,7,8-TCDD internal standard peak.	10	
b. The ion current responses for m/z 258.930, 319.897 and 321.894 must reach a maximum simultaneously (±1 second) and must be $\geq$2.5 times noise level.	10	
c. The m/z 319.897/321.894 ratio is $\geq$0.67 and $\leq$0.90.	10	
d. The m/z 331.937/333.934 ratio is $\geq$0.67 and $\leq$0.90.	5	
e. The S/N ratio for m/z 331.937 and 333.934 is $\geq$2.5.	5	
5. Identification Criteria for other TCDDs:		
a. Retention time must fall into window established by GC performance check.	5	
b. The ion current responses for m/z 258.930, 319.897, and 321.894 reach a maximum simultaneously (±1 second) and are $\geq$2.5 times noise level.	10	
c. The m/z 319.897/321.894 ratio is $\geq$0.67 and $\leq$0.90.	5	

Figure 1. (Continued).

		Possible Score	Score Achieved
6.	Concentrations of unlabeled TCDDs are calculated according to D-13.1.	10	
7.	Duplicate analysis values agree within ±50%.	10	
8.	Estimated detection limits calculated according to D-13.5.	10	
9.	Percent recovery of $^{13}C_{12}$-2,3,7,8-TCDD $\geq$40 and $\leq$120%.	10	
10.	Results documented with appropriate SIM traces and calculations.	20	
	Subtotal III	185	
	Total	290	

Figure 1. (Continued).

EVENT SEQUENCE FOR ON-SITE LABORATORY EVALUATION

I. Meeting with Laboratory Manager and Project Manager

Introduction; discuss purpose of visit; discuss problems with data submitted by the laboratory.

II. Verification of Personnel

Review qualification of contractor personnel in place and committed to project (Section I, SES).

III. Verification of Instrumentation

Review equipment in place and committeed to project (Section II, SES). The Contractor must demonstrate adequate equipment redundancy, as defined in SES, Section II.D., to ensure his capability to perform the required analyses in the required time.

IV. Quality Control Procedures

Walk through the laboratory to review:

1. Sample receiving and logging procedures,
2. Sample and extract storage area,
3. Procedures to prevent sample contamination,
4. Security procedures for laboratory and samples,
5. Safety procedures,
6. Conformance to written SOPs,
7. Instrument records and logbooks,
8. Sample and data control systems,
9. Procedures for handling and disposing of hazardous materials,
10. Glassware cleaning procedures,
11. Status of equipment and its availability,
12. Technical and managerial review of laboratory operations and data package preparations,
13. Procedures for data handling, analysis, reporting and case file preparation, and
14. Chain-of-custody procedures.

V. Review of Standard Operating Procedures (SOPs)

Review SOPs with the Project Manager to assure that the laboratory understands the dimensions and requirements of the program.

VI. Identification of Needed Corrective Actions

Discuss with the Project Manager the actions needed to correct weaknesses identified during the site inspection, PE sample analysis or production of

Figure 2. Event Sequence for On-Site Laboratory Evaluation.

reports (hard copies and, if appropriate, manual calculations) and documentation. Determine how and when corrective actions will be documented, how and when improvements will be demonstrated, and identify the contractor employee responsible for corrective actions.

VII. Previously Identified Problems

Check the most recent SES to verify that all previously identified problems have been corrected.

VIII. Identification of New Problems

a. Discuss any weaknesses identified in the performance evaluation sample analyses and reports.

b. Discuss any weaknessess identified in this site inspection.

Figure 2. (Continued).

SITE EVALUATION SHEET

Laboratory: ______________________________ Date: __________

Location: ______________________________________

EVALUATORS

Name	Organization
1.	
2.	
3.	
4.	
5.	
6.	
7.	

I. Laboratory Personnel Committed to Project:

A. Project Manager (responsible for overall technical effort)

Name: ______________________________________

Title: ______________________________________

B. GC/MS Operator: ______________________________

Experience:* ______________
(one year minumum)

C. GC/MS Data Interpreter: ______________________________

Experience:* ______________
(two year minimum)

D. Person responsible for sample exraction, column chromatography and extract concentration: ______________________

Experience:* ______________
(one year minumum)

E. Person(s) responsible for calculations and report preparation:

Hardcopy Reports: ______________________________

F. Person responsible for handling, storage and (if appropriate) preparation of solutions of standard compounds:

____

*Experience is deemed to mean "more than 50 percent of the person's productive work time."

Figure 3. Site Evaluation Sheet.

G. Person responsible for standards preparation/storage:

H. Person responsible for record keeping:

I. Quality Assurance Officer:

J. Personnel checklist

1. Do personnel assigned to this project have the appropriate level and type of experience to successfully accomplish the objectives of this program? ( ) Yes ( ) No

2. Is the organization adequately staffed to meet project requirements in a tmely manner? ( ) Yes ( ) No

3. Does the Laboratory Quality Assurance officer report to senior management levels? ( ) Yes ( ) No

4. Was the Quality Assurance officer available during the evaluation? ( ) Yes ( ) No

II. Laboratory Equipment

A. Gas chromatograph(s)*

Manufacturer and Model:
Installation Date:
Type of Capillary Column Injection System:
Capillary Column to be used (length, ID, coating, etc.):
Necessary Ancillary Equipment (gases, syringes, etc.):

B. High Resolution Mass Spectrometer(s)*

Static Resolution Capability (10,000 min.):
Peak matching system:
Manufacturer and Model:
Installation Date:
Pertinent Modifications:
Peak Matching System/Accuracy (Mfg. spec.):

C. Data System(s)*
Manufacturer and Model:

* If more than one GC/MS/DC, indicate system 1,2,3, etc., by numbering components with 1,2,3, etc.

Figure 3. (Continued).

Installation Date: ______________________________

Software Version Identifier: ______________________________

Appropriate selected ion monitoring software/hardware ( ) Yes ( ) No

Capability to produce hard copies of computer-generated information ( ) Yes ( ) No

D. Evidence that at least one GC/MS/DS system can be reasonably expected to be operating acceptably at any given time:

( ) More than one adequate GC/MS/DS system is available in-house, (i.e.,meeting requirements specified in SOW Section 6.1, Exhibit D).

( ) Appropriate in-house replacement parts and trained service personnel are available.

( ) A service contract is in place with guaranteed response time (specify type of contract and limitations). ______________________

( ) Voltage control devices are used on major instruments; isolated circuits are used.

( ) Other (specify) ______________________________

III. Facilities Checklist

A. Does the laboratory appear to have adequate workspace (120 sq. feet, 6 linear feet of unencumbered bench space per analyst)? ( ) Yes ( ) No

B. Does the laboratory have a source of distilled/demineralized water? ( ) Yes ( ) No

C. Is the analytical balance located away from draft and areas subject to rapid temperature changes or vibration? ( ) Yes ( ) No

D. Has the balance been calibrated within one year by a certified technician? ( ) Yes ( ) No

E. Is the balance routinely checked with class S weights before each use and the results recorded in a logbook? ( ) Yes ( ) No

F. Is the laboratory maintained in a clean and organized manner? ( ) Yes ( ) No

Figure 3. (Continued).

G. Is the facility designed for hazardous organic chemical analysis? ( ) Yes ( ) No

  1. Is ventilation provided in the sample preparation areas? ( ) Yes ( ) No

  2. Are vented hoods available and adequately vented in the sample preparation areas? ( ) Yes ( ) No

  3. Are the hoods equipped with charcoal and HEPA filters? ( ) Yes ( ) No

  4. Are instruments, including GC/MS pumps, vented into hoods or control devices such as charcoal traps? ( ) Yes ( ) No

H. Are adequate secured facilities provided for storage of samples, extracts, and calibration standards, including cold storage? ( ) Yes ( ) No

I. Are the temperatures of the cold storage units recorded daily in logbooks? ( ) Yes ( ) No

J. Are chemical waste disposal policies/procedures in place? ( ) Yes ( ) No

K. Is the laboratory secure? ( ) Yes ( ) No

IV. Analysis Control Checklist

A. Do the project personnel have SOPs for the required activities? ( ) Yes ( ) No

B. Is a logbook maintained for each instrument and is information such as calibration data and instrument maintenance continually recorded? ( ) Yes ( ) No

C. Do the analysts record bench data in a neat and accurate manner? ( ) Yes ( ) No

D. Standards

  1. Are fresh analytical standards prepared at a frequency consistent with good QC? ( ) Yes ( ) No

  2. Are reference materials properly labeled with concentrations, date of preparation, and the identity of the person preparing the sample? ( ) Yes ( ) No

  3. Is a standards preparation and tracking logbook maintained? ( ) Yes ( ) No

Figure 3. (Continued).

4. Are working standards traceable to EPA standards or validated against EPA standards? ( ) Yes ( ) No

V. Documentation/Tracking Checklist

A. Is a sample custodian designated? If yes, name of sample custodian. ( ) Yes ( ) No
Name: ______________________________

B. Are the sample custodian's procedures and responsibilities documented? If yes, where are these documented? ( ) Yes ( ) No

Are the chain-of-custody procedures documented? ( ) Yes ( ) No

C. Are written Standard Operating Procedures (SOPs) developed for receipt of samples? If yes, where are the SOPs documented (laboratory manual, written instructions, etc.)? ( ) Yes ( ) No

D. Are quality assurance procedures documented and available to the analysts? If yes, where are these documented? ( ) Yes ( ) No

E. Are written Standard Operating Procedures (SOPs) developed for compiling and maintaining sample document files? If yes, where are the SOPs documented (laboratory manual, written instructions, etc.)? ( ) Yes ( ) No

F. Are the magnetic tapes stored in a secure area? ( ) Yes ( ) No

G. Are samples that require preservation stored in such a way as to maintain their integrity? If yes, how are the samples stored? ( ) Yes ( ) No

Documentation/Notebooks Checklist

A. Is a permanently bound notebook with preprinted, consecutively numbered pages being used? ( ) Yes ( ) No

B. Is the type of work clearly displayed on the notebook? ( ) Yes ( ) No

C. Is the notebook maintained in a legible manner? ( ) Yes ( ) No

D. Are entries noting anomalies routinely recorded? ( ) Yes ( ) No

Figure 3. (Continued).

E. Has the analyst avoided obliterating entries or the use of a pencil? ( ) Yes ( ) No

F. Are inserts (i.e. chromatograms, computer print-outs, etc.) permanently affixed to the notebook and signed across insert edge and page? ( ) Yes ( ) No

G. Has the supervisor of the individual maintaining the notebook personally examined and reviewed the notebook periodically, and signed his/her name therein, together with the date and appropriate comments as to whether or not the notebook is being maintained in an appropriate manner? ( ) Yes ( ) No

H. Where applicable, is the notebook holder referencing reports or memoranda pertinent to the contents of an entry? ( ) Yes ( ) No

VI. Quality Control Manual Checklist

Does the laboratory maintain a Quality Assurance/ Quality Control (QA/QC) Manual? ( ) Yes ( ) No

Does the manual address the important elements of a QA/QC program, including the following: ( ) Yes ( ) No

A. Personnel ( ) Yes ( ) No

B. Facilities and equipment ( ) Yes ( ) No

C. Operation of instruments ( ) Yes ( ) No

D. Documentation of Procedures ( ) Yes ( ) No

E. Procurement and inventory practices ( ) Yes ( ) No

F. Preventive maintenance ( ) Yes ( ) No

G. Reliability of data ( ) Yes ( ) No

H. Data validation ( ) Yes ( ) No

I. Feedback and corrective action ( ) Yes ( ) No

J. Instrument calibration ( ) Yes ( ) No

K. Recordkeeping ( ) Yes ( ) No

L. Internal audits ( ) Yes ( ) No

Figure 3. (Continued).

Are QA/QC responsibilities and reporting relationships clearing defined? ( ) Yes ( ) No

Have standard curves been adequately documented? ( ) Yes ( ) No

Are laboratory standards traceable? ( ) Yes ( ) No

Are quality control charts maintained for each routine analysis? ( ) Yes ( ) No

Do QC records show corrective action when analytical results fail to meet QC criteria? ( ) Yes ( ) No

Do supervisory personnel review the data and QC results? ( ) Yes ( ) No

VII. Data Handling Checklist

Are data calculations checked by a second person? ( ) Yes ( ) No

Are data calculations documented? ( ) Yes ( ) No

Do records indicate corrective action that has been taken on projected data? ( ) Yes ( ) No

Are limits of detection determined and reported properly? ( ) Yes ( ) No

Are all data and records retained for the required amount of time? ( ) Yes ( ) No

Are quality control data (e.g., standard curve duplicates) accessible for all analytical results? ( ) Yes ( ) No

VIII. Summary

Do responses to the evaluation indicate that project and supervisory personnel are aware of QA/QC and its application to the project? ( ) Yes ( ) No

Do project and supervisory personnel place positive emphasis on QA/QC? ( ) Yes ( ) No

Have responses with respect to QA/QC aspects of the project been open and direct? ( ) Yes ( ) No

Has a cooperative attitude been displayed by all project and supervisory personnel? ( ) Yes ( ) No

Figure 3. (Continued).

Does the organization place the proper emphasis on quality assurance?	( ) Yes	( ) No
Have any QA/QC deficiencies been discussed before leaving?	( ) Yes	( ) No
Is the overall quality assurance adequate to accomplish the objectives of the project?	( ) Yes	( ) No
Have corrective actions recommended during previous evaluations been implemented?	( ) Yes	( ) No
Are any corrective actions required? If so, list the necessary actions below.	( ) Yes	( ) No